Meiotic S-Phase Damage Activates Recombination without Checkpoint Arrest[□]

Daniel G. Pankratz^{*†‡} and Susan L. Forsburg^{*§}

^{*}Molecular & Cell Biology Laboratory, The Salk Institute, La Jolla, CA 92037; [†]Division of Biology, University of California, San Diego, La Jolla, CA 92093-0346; and [§]Molecular and Computational Biology Section, University of Southern California, Los Angeles, CA 90089-1340

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Checkpoints operate during meiosis to ensure the completion of DNA synthesis and programmed recombination before the initiation of meiotic divisions. Studies in the fission yeast *Schizosaccharomyces pombe* suggest that the meiotic response to DNA damage due to a failed replication checkpoint response differs substantially from the vegetative response, and may be influenced by the presence of homologous chromosomes. The checkpoint responses to DNA damage during fission yeast meiosis are not well characterized. Here we report that DNA damage induced during meiotic S-phase does not activate checkpoint arrest. We also find that in wild-type cells, markers for DNA breaks can persist at least to the first meiotic division. We also observe increased spontaneous S-phase damage in checkpoint mutants, which is repaired by recombination without activating checkpoint arrest. Our results suggest that fission yeast meiosis is exceptionally tolerant of DNA damage, and that some forms of spontaneous S-phase damage can be repaired by recombination without activating checkpoint arrest.

INTRODUCTION

Meiosis is the specialized differentiation process that generates recombinant haploid gametes from a diploid zygote. Meiosis is initiated by a round of DNA synthesis (meiotic S-phase), followed by programmed homologous recombination in prophase and two sequential rounds of chromosome segregation. Meiotic recombination is required to generate recombinant progeny and ensure the accurate segregation of homologous chromosomes in the first meiotic division (MI) (Roeder, 1997). Recombination is initiated by DNA double strand breaks (DSBs) generated in replicated DNA by homologues of Spo11 (Keeney, 2001) and preferentially involve homologous chromosomes over sister chromatids (Schwacha and Kleckner, 1997). Thus, an unusual feature of meiosis is the deliberate generation and repair of DNA damage.

Conserved checkpoint responses recognize aberrant DNA structures and delay or arrest cell cycle progression until DNA breaks are repaired (Hartwell and Weinert, 1989; Weinert, 1998b; Nyberg *et al.*, 2002). While even a single DNA break can activate checkpoint-mediated arrest (Sandell and Zakian, 1993), DNA breaks form normally during DNA replication and the threshold for checkpoint activation during S-phase is elevated relative to other stages of the cell cycle (Shimada *et al.*, 2002a; Sogo *et al.*, 2002). It is not clear whether meiotic DNA damage checkpoint thresholds are

similarly elevated, either during meiotic S-phase or later in prophase when meiotic DSBs form.

Two DNA checkpoints have been identified in yeast meiosis. The first is a meiotic replication checkpoint (Murakami and Nurse, 1999), similar to the vegetative S-phase replication checkpoint, which stabilizes replication forks (Lopes *et al.*, 2001; Tercero and Diffley, 2001) and restrains the firing of late replication origins (Santocanale and Diffley, 1998; Shirahige *et al.*, 1998) in response to DNA damage (Paulovich and Hartwell, 1995) or nucleotide depletion by the ribonucleotide inhibitor hydroxyurea (HU) (Zhao *et al.*, 1998) during S-phase. In the fission yeast *Schizosaccharomyces pombe*, both the vegetative and meiotic replication checkpoints are dependent on *cds1*⁺ (ScRAD53) (Murakami and Okayama, 1995; Boddy *et al.*, 1998; Rhind and Russell, 1998) and the “checkpoint rad” genes *rad1*⁺, *rad3*⁺, *rad9*⁺, *hus1*⁺, *rad17*⁺, and *rad26*⁺ (al-Khodairy and Carr, 1992; Enoch *et al.*, 1992; Lindsay *et al.*, 1998; Murakami and Nurse, 1999).

Vegetative wild-type cells treated with HU arrest the cell cycle, and recover and complete replication when the drug is removed. In contrast, $\Delta cds1$ cells treated with HU undergo a lethal arrest in S-phase (Murakami and Okayama, 1995; Lindsay *et al.*, 1998), while checkpoint *rad* mutants fail to arrest and enter an aberrant mitosis without completing DNA replication (Enoch *et al.*, 1992). The failure of the replication checkpoint in vegetative $\Delta cds1$ cells exposed to HU causes DNA breaks as a consequence of replication fork collapse (Lopes *et al.*, 2001), activating the *chk1*⁺ kinase (Lindsay *et al.*, 1998). Chk1 is normally activated in a checkpoint rad-dependent manner in response to DNA damage in G2 phase of the cell cycle, preventing entry into mitosis (al-Khodairy *et al.*, 1994; Walworth and Bernards, 1996; Martinho *et al.*, 1998).

In contrast to the cell cycle, $\Delta cds1$ diploid cells blocked in meiosis with HU proceed with meiotic divisions after only a short delay, indistinguishable from diploid checkpoint *rad*

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[†] Present address: Oregon Health & Science University, NRC3, 3181 SW Sam Jackson Park Road, Portland, OR 97239.

Address correspondence to: Susan L. Forsburg (forsburg@usc.edu).

mutants under similar conditions (Murakami and Nurse, 1999). This difference from the behavior of vegetative cells suggests that the *chk1*⁺-dependent response to replication fork collapse may be attenuated or inactive during meiosis (Murakami and Nurse, 1999). Curiously, haploid Δ *cds1* and Δ *rad3* cells induced to enter meiosis undergo prolonged arrest when treated with HU (Forsburg and Hodson, 2000). This raises the possibility that the absence of homologous chromosomes triggers an alternative *rad3*⁺- and *cds1*⁺-independent checkpoint, perhaps involving Chk1 or other kinases, in response to the DNA damage caused by the failure of the replication checkpoint.

The second characterized meiotic checkpoint is a recombination checkpoint that monitors defects in the processing of meiotic recombination intermediates in pachytene (Roeder and Bailis, 2000). Fission yeast defective in homolog pairing undergo a modest delay in prophase (Nabeshima *et al.*, 2001). This delay, which can also be induced by ionizing radiation (IR) in prophase (Shimada *et al.*, 2002b), is *cds1*⁺, *mek1*⁺, and checkpoint *rad* dependent (Shimada *et al.*, 2002b; Perez-Hidalgo *et al.*, 2003). However, fission yeast cells completely deficient for recombination repair can still proceed with meiotic divisions (Catlett and Forsburg, 2003), suggesting that the recombination checkpoint response in fission yeast does not permanently arrest meiotic divisions in the presence of DNA damage. This is in contrast to budding yeast recombination and homolog pairing mutants, which arrest permanently in prophase in a *ScMEK1*-dependent manner (Lydall *et al.*, 1996; Xu *et al.*, 1997), and suggests that fission yeast meiosis is particularly tolerant of DNA damage.

To investigate checkpoint responses to DNA damage during fission yeast meiosis in greater detail, we examined the effects of DNA damage from a variety of sources on meiotic progression in wild-type and checkpoint deficient fission yeast strains. We show that DNA damage during meiotic S-phase does not delay meiosis in a checkpoint-dependent manner. We also show that Cds1 and Chk1 proteins are not additionally phosphorylated in response to DNA damage early in meiosis. Further, we find that fission yeast can assemble MI spindles in the presence of Rhp51 foci, confirming that certain forms of ongoing repair do not cause arrest in meiotic prophase, in contrast to observations in budding yeast (Lydall *et al.*, 1996). In addition, we observe that even unperturbed Δ *cds1* strains sustain elevated levels of spontaneous meiS-phase damage, which is adequately repaired by recombination before the completion of meiosis, suggesting that ongoing damage repair can occur during meiosis in the absence of checkpoint arrest. Taken together, these results show that damage thresholds for checkpoint activation in fission yeast meiosis are substantially elevated relative to thresholds during the cell cycle, such that cells do not delay or arrest their meiotic divisions in response to DNA damage.

MATERIALS AND METHODS

Strains and Media

Strains (Table 1) were constructed and maintained on yeast extract plus supplements (YES) or Edinburgh minimal medium (EMM) using standard techniques (Moreno *et al.*, 1991). Matings were performed on synthetic sporulation agar (SPAS) (Gutz *et al.*, 1974). *Rad3ts* strains were derivatives of TC1669 and TC1670 (Martinho *et al.*, 1998). Chk1-HA strains were derivatives of NW223 (Walworth and Bernards, 1996). Δ *rec12* strains were derivatives of GP1456 and GP1459 (Lin and Smith, 1994).

Synchronous Meiosis

Synchronous haploid meiosis was performed in the *pat1-114* genetic background essentially as described (Iino and Yamamoto, 1985b, a), except that starved cultures were refed at 34°C in the presence or absence of hydroxyurea

(HU) (Sigma), methylmethane sulfonate (MMS) (Sigma) or camptothecin (CPT) (Calbiochem) as appropriate. FACS analysis and nuclei counts were performed as described (Forsburg and Hodson, 2000). Haploid strains used in meiotic timecourses were as follows: wild-type (FY2057), *Acds1* (FY2182), Δ *chk1* (FY2184), Δ *rad3* (FY2186), Δ *tel1* (FY2188), Δ *mek1* (FY2386), Δ *cds1* Δ *mek1* (FY2806), Δ *tel1* Δ *mek1* (FY2807), *rad3ts* (FY2808), *rad3ts* Δ *tel1* (FY2809).

For synchronous diploid meiosis, we generated diploid strains expressing *mat1-Pc*, *mat1-Mc*, and *mat1-Mi*, but not *mat1-Pi*, in the *pat1-114* background using the *mat2-102* allele (Kelly *et al.*, 1988; Willer *et al.*, 1995; Yamamoto and Hiraoka, 2003). The following haploid strains were cross-ed to generate fresh diploids for each timecourse: FY2004 $\times$ FY2057 (wild-type), FY2245 $\times$ FY2182 (Δ *cds1*), FY2246 $\times$ FY2184 (Δ *chk1*), FY2247 $\times$ FY2186 (Δ *rad3*), FY2248 $\times$ FY2188 (Δ *tel1*), and FY2322 $\times$ FY2386 (Δ *mek1*). Strains were cross-ed on SPAS for 12–16 h at 25°C and selected for intragenic complementation at 25°C on EMM plates lacking supplemental adenine (EMM -ade). White *ade*⁺ colonies were streaked to YES + 5 μ g/ml phloxin B and incubated 3–5 d at 25°C. Starter cultures in liquid EMM -ade were inoculated with large pink colonies and incubated 2–3 d at 22–25°C. 300 μ l of cells were fixed in 70% ethanol and analyzed by FACS as described (Liang *et al.*, 1999). Cultures with prominent 2C and 4C peaks and a minimum of sub-1C particles were used to inoculate 10–150 ml batch cultures in EMM -ade. Meiotic timecourses were performed as described for haploid meiosis (Forsburg and Hodson, 2000), except that EMM -ade is used for washes and overnight starvation.

Integration of Cds1-HA

A Cds1 expression construct C-terminally tagged with hemeagglutinin (3 $\times$ HA) (gift of G. Brown) was excised by *XhoI* and *SalI* digestion from pSLF272 (Forsburg and Sherman, 1997) and cloned into the *XhoI* and *SalI* sites of pJK210 (Keeney and Boeke, 1994). The construct was linearized in Cds1 by *NheI* digestion and transformed into FY527 (*h*-*his3-D1 ura4-D18 leu1-32 ade6-M216*) at the endogenous locus by electroporation (Kelly *et al.*, 1993) to produce FY2244 (Table 1). Single copy insertion in *ura*⁺ transformants was confirmed by Southern blot and protein expression confirmed by western blot (not shown).

Protein Extraction and Western Blot Analysis

Diploid strains for analysis of Cds1 and Chk1 phosphorylation during mitosis and synchronous meiosis were generated from the following parental strains: FY2812 $\times$ FY3117 (Cds1-HA), FY2812 $\times$ FY2371 (Chk1-HA), FY2245 $\times$ FY2856 (Δ *cds1* Chk1-HA). Proteins were isolated by denaturing trichloroacetic acid extraction (Caspari *et al.*, 2000) as described (Catlett and Forsburg, 2003). 7.5 μ g (Cds1-HA strains) or 15 μ g (Chk1-HA strains) total protein was separated on 10% SDS-PAGE gels run for 16 h at 4°C. Proteins were transferred to Immobilon-P membrane in 10 mM CAPS pH 11, 10% methanol for 1 h at 45V, blocked for 1 h at room temperature in Tris-buffered saline + 1% Tween 20 + 5% nonfat milk and HA-tagged proteins detected by overnight incubation with mouse anti-HA antibody HA.11 (Covance) at 1:2500 dilution. PCNA was detected using mouse anti-PCNA antibody PC10 (Santa Cruz Biotechnology) at 1:2000 dilution. Primary antibodies were detected by overnight incubation with HRP-conjugated anti-mouse secondary antibody (Jackson ImmunoResearch) at 1:2000 dilution followed by chemiluminescent detection (ECL+, Amersham).

Immunofluorescence

Synchronous meiotic timecourses were sampled and meiotic chromosome spreads prepared as described (Hodson *et al.*, 2003). Heat-fixed spread preparations on glass slides were blocked for 1 h in 100 μ l phosphate-buffered saline (PBS)/10% calf serum/0.05% NaN₃ (PCA) at room temperature under coverslips in a humidified chamber, followed by overnight incubation with either rabbit polyclonal antiphospho-H2A.X (Upstate) at 1:50 dilution or rabbit polyclonal anti-Rhp51 (Catlett and Forsburg, 2003) at 1:100 dilution in PCA. Slides were washed briefly in PBS followed by incubation for two hours with chicken anti-rabbit Alexa Fluor 488 (Molecular Probes) at 1:250 dilution in PCA. Slides were washed again briefly in PBS, air-dried vertically in the dark, and mounted in medium (1 mg/ml p-phenylenediamine, 1 mg/ml n-propyl gallate, 10% PBS, 90% glycerol, pH9.0) + DAPI (0.66 μ l of 1 mg/ml DAPI in dimethyl sulfoxide per ml of mount medium) under coverslips sealed with nail polish. Immunofluorescence on spreads was visualized using a Leica DMR microscope with a Hamamatsu CCD camera and images were captured using Openlab (Improvision) software.

For MI spindle assembly assays, 5 $\times$ 10⁶ diploid cells fixed in 70% ethanol were washed twice in 500 μ l PBS with gentle spinning (1000g for 2 min) and resuspended by inversion in 0.5 mg/ml Zymolyase 20T in PBS. Cells were incubated for 10 min at room temperature, checked for spheroplast formation, washed twice in PBS and pipetted onto poly-L-lysine coated glass slides. Cells were incubated for 10 min, supernatant removed by pipetting and adherent spheroplasts heat fixed at low heat until remaining liquid evaporated (1–2 min). Slides were blocked for 1 h in PCA and incubated simultaneously overnight with mouse monoclonal antitubulin antibody 4A1 (a kind gift of Richard McIntosh) at 1:50 dilution and rabbit polyclonal anti-Rhp51 at 1:500 dilution in PCA. Slides were washed in PBS and incubated for 2 h with chicken anti-mouse Alexa Fluor 594 (Molecular Probes) and goat anti-rabbit

Table 1. Strains used in this study

Strain	Genotype	Source
FY12	<i>h+ ade6-M210</i>	Laboratory stock
FY13	<i>h- ade6-M216</i>	Laboratory stock
FY2004	<i>mat2-102 pat1-114 ura4-D18 ade6-M210</i>	Laboratory stock
FY2057	<i>h- pat1-114 ade6-M216</i>	Laboratory stock
FY2182	<i>h- pat1-114 Δcds1::ura4+ ura4 ade6-M216</i>	This work
FY2184	<i>h- pat1-114 Δchk1::ura4+ ura4-D18 ade6-M216</i>	This work
FY2186	<i>h- pat1-114 Δrad3::ura4+ ura4-D18 ade6-M216</i>	This work
FY2188	<i>h- pat1-114 Δtel1::ura4+ ura4-D18 ade6-M216</i>	This work
FY2189	<i>h+ Δcds1::ura4+ ura4 ade6-M216</i>	This work
FY2190	<i>h- Δcds1::ura4+ ura4 ade6-M216</i>	This work
FY2191	<i>h+ Δchk1::ura4+ ura4-D18 ade6-M216</i>	This work
FY2192	<i>h- Δchk1::ura4+ ura4-D18 ade6-M216</i>	This work
FY2193	<i>h+ Δrad3::ura4+ ura4-D18 ade6-M216</i>	This work
FY2194	<i>h- Δrad3::ura4+ ura4-D18 ade6-M216</i>	This work
FY2195	<i>h+ Δtel1::ura4+ ura4-D18 ade6-M216</i>	This work
FY2196	<i>h- Δtel1::ura4+ ura4-D18 ade6-M216</i>	This work
FY2244	<i>h- cds1-HA::ura4+ ura4-D18 his3-D1 leu1-32 ade6-M216</i>	This work
FY2245	<i>mat2-102 pat1-114 Δcds1::ura4+ ura4 ade6-M210</i>	This work
FY2246	<i>mat2-102 pat1-114 Δchk1::ura4+ ura4-D18 ade6-M210</i>	This work
FY2247	<i>mat2-102 pat1-114 Δrad3::ura4+ ura4-D18 ade6-M210</i>	This work
FY2248	<i>mat2-102 pat1-114 Δtel1::ura4+ ura4-D18 ade6-M210</i>	This work
FY2314	<i>h- ade6-L469/pUC8/his3+/ade6-M375 his3-D1</i>	This work
FY2321	<i>h+ ade6-M375-M210 his3-D1</i>	This work
FY2322	<i>mat2-102 pat1-114 Δmek1::his3+ his3-D1 ade6-M210</i>	This work
FY2371	<i>h- pat1-114 chk1-HA ade6-M216</i>	This work
FY2376	<i>h- Δcds1::ura4+ ura4 ade6-L469/pUC8/his3+/ade6-M375 his3-D1</i>	This work
FY2377	<i>h- Δchk1::ura4+ ura4-D18 ade6-L469/pUC8/his3+/ade6-M375 his3-D1</i>	This work
FY2378	<i>h- Δrad3::ura4+ ura4-D18 ade6-L469/pUC8/his3+/ade6-M375 his3-D1</i>	This work
FY2379	<i>h- Δtel1::ura4+ ura4-D18 ade6-L469/pUC8/his3+/ade6-M375 his3-D1</i>	This work
FY2386	<i>h- pat1-114 Δmek1::his3+ his3-D1 ade6-M216</i>	This work
FY2479	<i>h+ Δcds1::ura4+ ura4 ade6-M375-M210 his3-D1</i>	This work
FY2480	<i>h+ Δchk1::ura4+ ura4-D18 ade6-M375-M210 his3-D1</i>	This work
FY2481	<i>h+ Δrad3::ura4+ ura4-D18 ade6-M375-M210 his3-D1</i>	This work
FY2482	<i>h+ Δtel1::ura4+ ura4-D18 ade6-M375-M210 his3-D1</i>	This work
FY2806	<i>h- pat1-114 Δcds1::ura4+ Δmek1::his3+ ura4 his3-D1 ade6-M216</i>	This work
FY2807	<i>h- pat1-114 Δtel1::ura4+ Δmek1::his3+ ura4 his3-D1 ade6-M216</i>	This work
FY2808	<i>h- pat1-114 rad3ts ade6-M216</i>	This work
FY2809	<i>h- pat1-114 rad3ts Δtel1::ura4+ ura4-D18 ade6-M216</i>	This work
FY2812	<i>mat2-102 pat1-114 ade6-M210</i>	This work
FY2856	<i>h- pat1-114 chk1-HA Δcds1::ura4+ ura4 ade6-M216</i>	This work
FY2925	<i>h+ Δcds1::ura4+ ura4 Δrec12::LEU2 leu1-32</i>	This work
FY2926	<i>h- Δcds1::ura4+ ura4 Δrec12::LEU2 leu1-32</i>	This work
FY2928	<i>h+ Δchk1::ura4+ ura4-D18 Δrec12::LEU2 leu1-32</i>	This work
FY2929	<i>h- Δchk1::ura4+ ura4-D18 Δrec12::LEU2 leu1-32</i>	This work
FY2931	<i>h+ Δrad3::ura4+ ura4-D18 Δrec12::LEU2 leu1-32</i>	This work
FY2932	<i>h- Δrad3::ura4+ ura4-D18 Δrec12::LEU2 leu1-32</i>	This work
FY3073	<i>h+ Δrec12::LEU2 leu1-32</i>	This work
FY3074	<i>h- Δrec12::LEU2 leu1-32</i>	This work
FY3077	<i>h+ Δtel1::ura4+ ura4-D18 Δrec12::LEU2 leu1-32</i>	This work
FY3078	<i>h- Δtel1::ura4+ ura4-D18 Δrec12::LEU2 leu1-32</i>	This work
FY3117	<i>h- pat1-114 cds1-HA::ura4+ ura4-D18 ade6-M216</i>	This work

Oregon Green 488 (Molecular Probes) at 1:250 dilution in PCA. Slides were washed in PBS, air-dried vertically in the dark, and mounted in mount medium + DAPI. Cells were visualized as described.

Recombination and Spore Viability Assays

Sister chromatid exchange (SCE) during meiosis was assayed at the *ade6* locus using the *ade6-L469/pUC8/his3+/ade6-M375* (Osman *et al.*, 2000) and *ade6-M375-M210* alleles as described (Catlett and Forsburg, 2003), except that spores were plated on YES to first assay viability and replica plated to EMM -ade to assay adenine prototrophy. Strains crossed to generate spores for the SCE assay were FY2314 and FY2321 (wild-type), FY2376 and FY2479 (*Δcds1*), FY2377 and FY2480 (*Δchk1*), FY2378 and FY2481 (*Δrad3*), and FY2379 and FY2482 (*Δtel1*). Spore viability assays were performed by tetrad dissection as described (Catlett and Forsburg, 2003), except spores were grown on YES for 5 d at 25°C following microdissection. Strains crossed for spore viability assays were FY12 and FY13 (wild-type), FY2189 and FY2190 (*Δcds1*), FY2191

and FY2192 (*Δchk1*), FY2193 and FY2194 (*Δrad3*), FY2195 and FY2196 (*Δtel1*), FY3073 and FY3074 (*Δrec12*), FY2925 and FY2926 (*Δcds1 Δrec12*), FY2928 and FY2929 (*Δchk1 Δrec12*), FY2931 and FY2932 (*Δrad3 Δrec12*), FY3077 and FY3078 (*Δtel1 Δrec12*). P values were calculated in Statview (SAS Institute) by unpaired *t* test; a *p*-value ≤0.05 is considered statistically significant.

RESULTS

The Meiotic Response to Replication Checkpoint Failure Is Influenced by the Ploidy of Cells

Previously published results showed that diploid *Δcds1* and *Δrad3* mutants treated with HU during meiosis will proceed with abnormal meiotic divisions after a short delay (Mura-kami and Nurse, 1999). In contrast, haploid *Δcds1* and

$\Delta rad3$ mutants treated with HU during meiosis show a prolonged arrest of divisions (Forsburg and Hodson, 2000). We speculated that this difference in arrest phenotype was due to the difference in ploidy of the strains tested (Forsburg, 2002). To test this, we repeated the meiotic replication block experiments using isogenic diploid and haploid strains undergoing synchronous, *pat1-114* driven meiosis (Iino and Yamamoto, 1985b, a) (Figure S1A). In agreement with the published work (Murakami and Nurse, 1999; Forsburg and Hodson, 2000), we observe that diploid $\Delta cds1$ and $\Delta rad3$ strains released into meiosis in the presence of HU showed a short, ~ 1 h delay before proceeding into abnormal meiotic divisions while $\Delta cds1$ and $\Delta rad3$ haploids under similar conditions reproducibly displayed a substantially prolonged delay of divisions (Figure S1B). To determine whether other known checkpoint proteins play a role in restraining meiotic divisions when homologues are absent, we examined meiotic progression in $\Delta mek1$ and $\Delta tel1$ mutants as well. We observed no substantial alterations in meiotic progression in $\Delta mek1$ and $\Delta tel1$ single mutant haploids (Figure S1C) or diploids (data not shown) relative to wild-type, in the presence or absence of HU. Furthermore, meiotic divisions in $\Delta cds1 \Delta mek1$, $rad3ts \Delta tel1$ (Figure S1C), or $\Delta tel1 \Delta mek1$ double mutant haploids (data not shown) exposed to HU are not accelerated relative to $\Delta cds1$ and $rad3ts$ single mutants, suggesting that Mek1 and Tel1 do not play a role in restraining meiotic progression in haploid fission yeast under conditions of replication checkpoint failure. Nevertheless, these results confirm our hypothesis that homologous chromosomes influence meiotic progression by an unknown mechanism under conditions of irreversible replication fork collapse. To further our understanding of the normal meiotic response to DNA damage, we next examined the response of meiotic diploids to DNA damage from a variety of sources, including damage induced by exogenous chemical agents, the failure of the replication checkpoint, and spontaneous damage arising during meiotic S-phase.

DNA Damage Does Not Induce a Checkpoint Dependent Delay of Meiotic Divisions

Vegetative $\Delta cds1$ cells treated with HU arrest cell cycle progression in a *chk1*⁺-dependent manner (Lindsay *et al.*, 1998), which contrasts with the lack of arrest observed in $\Delta cds1$ diploids treated with HU during meiosis (Murakami and Nurse, 1999) (Figure S1). This suggests that the *chk1*⁺-dependent response to DNA damage caused by the collapse of replication forks (Lopes *et al.*, 2001) may be attenuated during fission yeast meiosis. To examine the meiotic response to other forms of DNA damage in S-phase, we examined meiotic progression in diploid cells exposed to exogenous, S-phase specific DNA damaging agents. The DNA topoisomerase I inhibitor camptothecin (CPT) causes double strand break (DSB) formation in the presence of active replication forks (Wan *et al.*, 1999). Wild-type cells released into synchronous meiosis in the presence of 10 μ M CPT, a concentration sufficient to activate Chk1 and block mitosis in cycling cells (Wan *et al.*, 1999), show only a slightly delayed entry into MI relative to untreated cells ($\sim 45\%$ mononucleates in CPT-treated cells at 5 h vs. $\sim 10\%$ mononucleate cells in untreated wild-type, Figure 1A). However, a substantial increase in aberrant chromosome segregation is observed under these conditions (Figure 1B), suggesting that treatment with CPT early in fission yeast meiosis causes persistent DNA damage to chromosomes. A similar delay of meiotic divisions occurred in $\Delta chk1$ mutants treated with CPT as well as $\Delta cds1$, $\Delta rad3$, $\Delta tel1$ and $\Delta mek1$ cells, relative to wild-

type (Figure 1A and data not shown). These data suggest that the minor CPT-induced meiotic delay we observe is not checkpoint-dependent, and that this level of CPT causes damage to chromosomes which results in aberrant segregation later in meiosis. Thus, DSB formation in S-phase induced by CPT does not restrain meiotic divisions in a checkpoint-dependent manner, suggesting that the DNA damage response to DSBs is attenuated during fission yeast meiosis.

The DNA alkylating agent methylmethane sulfonate (MMS) slows progression through vegetative S-phase in a checkpoint-independent manner and causes S-phase dependent lethality in replication checkpoint mutants (Tercero and Diffley, 2001). Checkpoint mutants released into synchronous meiosis in the presence of 0.01% MMS undergo delayed, abnormal meiotic divisions at a similar rate ($\Delta chk1$ and $\Delta tel1$) or slightly accelerated rate ($\Delta cds1$ and $\Delta rad3$) relative to wild-type (Figure 1A and data not shown). Accelerated divisions in $\Delta cds1$ or $\Delta rad3$ cells is accompanied by accelerated DNA synthesis in MMS in these mutants, as assayed by FACS analysis (data not shown); this may be due to the inappropriate firing of late origins in these mutants (Tercero and Diffley, 2001) and makes the accelerated divisions we observe under these conditions (Figure 1A) difficult to interpret. Nevertheless, these data suggest that some forms of DNA damage can cause a delay of meiotic divisions in fission yeast. However, the extent of the delay appears dependent on the nature and extent of the damage and occurs independently of a classic checkpoint arrest response.

Chromosome Missegregation Induced by DNA Damage Is not Elevated in Checkpoint Mutants

In addition to preventing cell division, checkpoints also activate repair (Weinert, 1998a; Nyberg *et al.*, 2002). If a meiotic checkpoint response normally activates the repair of damage without delaying meiotic divisions, then a repair defect in checkpoint mutants might result in increased aberrant chromosome segregation as mutant cells enter divisions with unrepaired lesions. To test this possibility, we examined the relative frequencies of aberrant nuclear morphology as compared with normal morphology ten hours after meiotic induction in checkpoint mutants and wild-type. In untreated cells, a modest increase in aberrant chromosome segregation is observed in checkpoint mutants relative to wild-type (Figure 1B), however checkpoint mutant spore viability is very similar to wild-type in untreated cells (Figure 4C), suggesting that this modest increase in aberrant chromosome segregation does not lead to a substantial loss of meiotic viability. Cells treated with CPT show elevated levels of aberrant segregation which occurs to a similar extent in checkpoint mutants and wild-type (Figure 1B), suggesting that checkpoint proteins are not important for promoting the repair of DSBs induced during meiotic S-phase by this drug. In contrast, MMS treated checkpoint mutants show a subtle increase in abnormal chromosome segregation relative to similarly treated wild-type cells (Figure 1B), suggesting that these checkpoint proteins might be important for either the prevention or repair of damage induced by MMS. While these data raise the possibility that checkpoint proteins may play a role in the activation of meiotic damage repair, the effect is subtle and occurs independent of a canonical checkpoint arrest response.

Cds1 and Chk1 Are not Activated by DNA Damage during Meiosis

Cds1 is activated in vegetative cells exposed to ionizing radiation in S-phase (Lindsay *et al.*, 1998) and is required for S-phase delay in response to UV (Rhind and Russell, 1998)

Figure 1. Checkpoint proteins do not arrest meiotic divisions in response to exogenous DNA damage. (A) Diploid cultures were released into synchronous meiosis in the presence or absence of camptothecin (CPT) or methylmethane sulfonate (MMS) and analyzed for meiotic progression. The proportion of cells having undergone no meiotic divisions (1 nucleus, circles), MI (2 nuclei, squares) or MII (3+ nuclei, triangles) were scored at various timepoints by DAPI microscopy. 300 cells were counted per timepoint. Divisions in $\Delta cds1$ cells are slightly accelerated relative to wild-type in the absence of DNA damage; $\Delta rad3$ cells behave similarly to $\Delta cds1$ cells (data not shown). (B) 300 cells from the ten hour timepoints shown in (A) were scored for normal and abnormal asci formation by DAPI microscopy and results represented as the percent average of total cells. (C) Cds1 and Chk1 protein phosphorylation in response to meiotic DNA damage was analyzed by anti-HA western blot. Diploids expressing Cds1-HA or Chk1-HA were sampled for protein during vegetative growth (asyn.), overnight G1 arrest (0 h timepoint), and 4 h after the induction of synchronous meiosis in the presence or absence of 5mU/ml bleomycin (bleo.), 0.005% MMS, or 15 mM hydroxyurea (HU). Chk1 protein from vegetative $\Delta cds1$ diploids in HU for 3 h and in meiosis for 5 h is included as a phosphorylation control, as well as Chk1 protein from vegetative wild-type cells treated with 0.1% MMS for one hour. CPT treated cells complete meiS by 3 h while HU treated cells remain arrested with 2C DNA content beyond 5 h by FACS (data not shown). PCNA levels are included as a loading control.

Figure 2. Meiotic cells assemble MI spindles in the presence of Rhp51 foci. Indirect immunofluorescence against α -tubulin and Rhp51 was performed on ethanol fixed diploid cells released into synchronous meiosis in the presence or absence of 15 mM hydroxyurea (+HU) or 0.01% methylmethane sulfonate (+MMS). Cells with assembled MI spindles (tubulin, red) were examined for the presence of Rhp51 foci (green) on DNA (DAPI, blue). Representative MI cells with (wild-type +MMS, $\Delta cds1$ +HU) and with-

out prominent Rhp51 foci (wild-type) are shown. A total of 100 cells with assembled MI spindles per condition from two independent blinded experiments were counted; percentages represent the average number of MI cells with prominent Rhp51 foci from both experiments. 34% of untreated $\Delta cds1$ cells and 39% of untreated $\Delta chk1$ cells with MI spindles showed Rhp51 foci. Scale bar = 10 μ m.

or MMS (Marchetti *et al.*, 2002). We examined Cds1 phosphorylation, a marker of Cds1 activation, in response to DNA damage during meiotic S-phase in wild-type diploids (Figure 1C). Cds1 protein levels increase and show a modest electrophoretic shift early in meiosis (Figure 1C), as previously reported (Shimada *et al.*, 2002b). We observe no additional shift in meiotic Cds1 mobility in response to levels of the radiomimetic drug bleomycin sufficient to arrest vegetative cells (Rhind and Russell, 2001) or levels of MMS sufficient to induce a ~1-h delay of meiotic S-phase (Figure 1C and data not shown) and aberrant chromosome segregation in wild-type meiotic cells (66% aberrant asci in 0.005% MMS vs. 12% in untreated cells). We observe substantially increased expression and phosphorylation of Cds1 in HU-treated meiotic cells relative to untreated meiotic cells (Figure 1C), consistent with a Cds1-dependent meiotic replication checkpoint response (Murakami and Nurse, 1999) (Figure S1). These results show that DNA damaging agents do not result in an increased expression or upregulation of Cds1 protein during meiosis analogous to that observed in response to HU.

We next monitored the electrophoretic mobility of Chk1 protein, which is activated by DNA damage in G2 phase (al-Khodairy *et al.*, 1994; Walworth and Bernardis, 1996; Martinho *et al.*, 1998) and in $\Delta cds1$ cells under replication block in S-phase (Lindsay *et al.*, 1998). There was very little shifted Chk1 visible in meiotic wild-type cells treated with bleomycin or MMS or in meiotic $\Delta cds1$ cells treated with HU (Figure 1C, right panel). In contrast, vegetative wild-type cells treated with MMS or vegetative $\Delta cds1$ cells treated with HU (Figure 1C; asyn. +MMS, $\Delta cds1$ asyn. +HU lanes) show a dramatic alteration in the mobility of a subset of Chk1 protein. Thus, Chk1 phosphorylation in response to DNA damage during meiosis appears severely attenuated relative to mitotic responses. These results are consistent with the absence of checkpoint-dependent delay of meiosis in response to damage (Figure 1A), and suggests that Cds1- and Chk1- dependent damage responses during fission yeast meiosis are insensitive to levels of damage sufficient to activate vegetative checkpoints.

Meiotic Divisions Can Occur in the Presence of Rhp51 Foci

If DNA damage during meiotic S-phase in *S. pombe* does not activate checkpoint delay, repair may not be initiated or completed before cells proceed with meiotic divisions. To test whether DNA damage induced in meiotic S-phase is repaired by recombination before chromosome segregation or persists through the first meiotic division (MI), we examined whether MI spindles assemble in the presence of Rhp51 foci during diploid meiosis. *rhp51*⁺ is the

fission yeast homolog of ScRAD51, which is a marker of single-stranded DNA intermediates in the repair of damage by homologous recombination (HR) (Lydall *et al.*, 1996; Caspari *et al.*, 2002). In *S. cerevisiae*, checkpoint mutants assemble MI spindles in the presence of Rad51 foci and undergo meiotic divisions in the presence of incomplete recombination while wild-type cells do not (Lydall *et al.*, 1996).

In marked contrast to observations in budding yeast, we observe that greater than 25% of wild-type *S. pombe* cells assemble MI spindles in the presence of Rhp51 foci, while 34% of untreated $\Delta cds1$ and 39% of untreated $\Delta chk1$ cells show Rhp51 foci on assembled MI spindles. More strikingly, >86% of wild-type cells undergoing meiosis in the presence of 0.01% MMS show Rhp51 foci in cells with assembled MI spindles, as well as the majority of $\Delta cds1$ cells in HU (Figure 2), indicating that the population of cells with Rhp51 foci on assembled spindles increases under conditions of DNA damage. We propose that fission yeast cells undergo at least the first meiotic division in the presence of DNA breaks or incomplete recombination structures as marked by Rhp51 foci. Our observation that the majority of cells are viable, despite the presence of Rhp51 foci at MI, suggests that damage is repaired subsequent to meiosis I or that Rhp51 foci do not represent actual DNA breaks. Nevertheless, these results strongly suggest that S-phase damage does not activate a checkpoint arrest either during S-phase or later, during meiotic prophase, and demonstrates that fission yeast meiosis is particularly tolerant of DNA damage.

cds1 and *rad3* Mutants Sustain Elevated Levels of Spontaneous Damage during Meiotic S-phase

Spontaneous DNA damage can occur upon passage through a normal S-phase (Zou and Rothstein, 1997). In *S. cerevisiae*, the *cds1*⁺ homolog RAD53 and the *rad3*⁺ homolog MEC1 are implicated in replication fork stability (Lopes *et al.*, 2001; Cha and Kleckner, 2002). Increased levels of Rad52 foci, a marker for DNA repair, are observed in *mec1* mutants during S and G2 phase (Lisby *et al.*, 2001), raising the possibility that endogenous levels of spontaneous S-phase DNA breaks might be elevated in replication checkpoint mutants. Therefore, we examined $\Delta cds1$ and $\Delta rad3$ cells during meiotic S-phase and prophase for DSB formation by indirect immunofluorescence against phosphorylated histone H2A, a marker of DNA breaks (reviewed in Pilch *et al.*, 2003). In *S. pombe*, the two H2A homologues Hta1 and Hta2 are phosphorylated in a *rad3*⁺- and *tel1*⁺-dependent manner in response to ionizing radiation, and are required for the maintenance of checkpoint arrest (Nakamura *et al.*, 2004). Phospho-H2AX formation in mammalian (Mahadevaiah *et al.*, 2001) and yeast meiosis (Perera *et al.*, 2004) has been

Figure 3. Replication checkpoint mutants sustain increased DNA damage during meiotic S-phase. (A) Immunofluorescence against phosphorylated histone H2A (γ H2AX, green) was performed on chromosome spreads prepared hourly from diploid cultures released into synchronous meiosis in the presence or absence of 15 mM hydroxyurea (HU). DAPI staining for DNA is shown in blue; phospho-H2A localizes to DNA (merge). Representative spreads with less (wild-type, 2 h) or more ($\Delta cds1$, 2 h) than 50% of visible DNA immunoreactive to anti- γ H2AX are shown. Scale bar = 10 μ m. (B) 100 spreads per timepoint from three independent timecourses were quantitated by blinded counts for phospho-H2A immunoreactivity. The average number of spreads with greater than 50% (stippled bars) or $\leq 50\%$ (white bars, stacked; height of both bars represents total percentage of immunoreactive spreads) of DNA area showing γ H2AX immunoreactivity is indicated at each timepoint; error bars = ± 1 SD. Approximately 20% of $\Delta cds1$ cells in an unperturbed meiotic S-phase show γ H2AX immunoreactivity analogous to what is observed in the majority of $\Delta cds1$ cells under replication block. The majority of cells in all three timecourses were in S-phase at 2 h, as determined by FACS analysis (data not shown).

correlated with Spo11 activity (Keeney *et al.*, 1997), suggesting meiosis-specific DSB formation corresponds to the formation of phospho-H2A domains.

We compared the fraction of cells with phospho-H2A immunoreactivity during normal meiotic S-phase and prophase in $\Delta cds1$, $\Delta rad3$, and wild-type strains (Figure 3). We found that 73% of chromosome spreads from $\Delta cds1$ cells in meiotic S-phase show phospho-H2A immunoreactivity compared with 44.6% in wild-type (Figure 3A and B, 2 h). Significantly, immunoreactive $\Delta cds1$ spreads tend to show larger domains of phospho-H2A phosphorylation than wild-type ($17.7\% \pm 4\%$ of spreads with $\leq 50\%$ area immunoreactive in $\Delta cds1$ vs. $0.3\% \pm 0.6\%$ in wild-type) (Figure 3B). These domains decrease in signal intensity after S phase is complete in both untreated wild-type and $\Delta cds1$ cells (Figure 3B, 3 h), suggesting repair during meiotic prophase. Similarly large domains of phospho-H2A immunoreactivity are also observed in a subset of spreads from untreated $\Delta rad3$ cells (Figure 3B). Because progression through meiosis is similar in wild-type and mutant strains (Figure 1 and data not shown), this suggests that an increased rate of DNA damage occurs in checkpoint mutants even during an unperturbed meiosis.

Under replication block in HU, $\Delta cds1$ and $\Delta rad3$ cells accumulate larger and more persistent phospho-H2A domains than wild-type (Figure 3A). This correlates with the irreversible replication fork collapse and DNA damage thought to occur in replication checkpoint mutants in HU (Lindsay *et al.*, 1998; Lopes *et al.*, 2001) and suggests that extensive Tel1-dependent H2A phosphorylation occurs during diploid meiosis. Thus, $\Delta cds1$ and $\Delta rad3$ cells accumulate increased levels of spontaneous DNA damage during a normal meiotic S-phase, as well as under replication block in HU.

Checkpoint Mutants Show Increased Meiotic Recombination

Recombination intermediates increase during S-phase, suggesting that S-phase DNA lesions can be repaired by recombination (Zou and Rothstein, 1997). Meiotic recombination, which is initiated by Spo11-dependent DSB formation (Keeney *et al.*, 1997), occurs preferentially between homologous chromosomes and is required for chromosome disjunction at MI (Schwacha and Kleckner, 1997). Radiation-induced DNA damage can partially rescue the viability defect in *spo11* mutants (Thorne and Byers, 1993; Dernburg *et al.*, 1998), suggesting that some forms of DNA damage in meiosis can become a substrate for meiotic recombination. Therefore, we reasoned that the elevated spontaneous S-phase breaks we observe in checkpoint mutants might be repaired in part by homologous recombination during meiosis.

To investigate this, we examined whether checkpoint mutants rescue spore viability in cells lacking the *S. pombe* *SPO11* homolog *rec12*⁺ (Sharif *et al.*, 2002). By tetrad dissection we observed a statistically significant elevation in spore viability in the progeny of $\Delta cds1 \Delta rec12$ ($52.5\% \pm 16.2\%$), $\Delta chk1 \Delta rec12$ ($48.6\% \pm 9.9\%$), and $\Delta rad3 \Delta rec12$ ($45.0\% \pm 5.9\%$) double mutants relative to $\Delta rec12$ single mutant parentals ($25\% \pm 6.8\%$); spore viability in $\Delta tel1 \Delta rec12$ double mutants ($30.3\% \pm 14.4\%$) is not statistically elevated relative to $\Delta rec12$ (Figure 4A). These data suggest that the spontaneous S-phase DNA breaks we observe in $\Delta cds1$ and $\Delta rad3$ checkpoint mutants can become a substrate of meiotic recombination, and that spontaneous DNA damage is also increased in $\Delta chk1$, but not $\Delta tel1$ mutants.

While meiotic recombination occurs preferentially between homologous chromosomes, an increase in ectopic sister chromatid exchange events is observed in some re-

Figure 4. Checkpoint mutants sustain increased meiotic recombination without loss of spore viability. (A) Reduced spore viability in a $\Delta rec12$ background is partially rescued by loss of checkpoint proteins. Checkpoint deficient $\Delta rec12$ parental strains were mated and microdissected asci assayed for colony formation on rich medium. Average viability (y-axis) in 80 asci from four independent cross-es was analyzed for each double mutant and $\Delta rec12$ single mutant progeny. Error bars = ± 1 SD. Viability is significantly elevated in $\Delta cds1 \Delta rec12$ (p-value = 0.021), $\Delta chk1 \Delta rec12$ (p-value = 0.008), and $\Delta rad3 \Delta rec12$ (p-value = 0.005) (asterisks) relative to $\Delta rec12$, whereas $\Delta tel1 \Delta rec12$ viability is not (p-value = 0.529). (B) Percent of *ade+* recombinant progeny (y-axis) recovered in cross-es of *ade-* checkpoint mutants; *ade* prototrophy is indicative of sister chromatid exchange (SCE). A minimum of 5600 viable spores from four independent cross-es of each checkpoint mutant and wild-type (wt) were assayed for *ade* prototrophy; error bars = ± 1 SD. SCE is statistically elevated in $\Delta tel1$ (p-value = 0.033) and $\Delta cds1$ (p-value = 0.017) (asterisks) but not $\Delta chk1$ (p-value = 0.146) or $\Delta rad3$ (p-value = 0.185) mutants relative to wild-type levels. (C) Spore viability as assayed by tetrad dissection onto rich media (50 asci per mutant) is listed as a percentage of theoretical maximum viability (four spores per ascus).

combination as well as checkpoint mutants during meiosis (Grushcow *et al.*, 1999; Catlett and Forsburg, 2003), suggesting either damage repair or a direct involvement of checkpoint proteins in regulating recombination partner choice (Grushcow *et al.*, 1999). We examined sister chromatid exchange (SCE) events at the *ade6* locus using a nontandem heteroallelic duplication (*ade6-M375int::pUC8/his3+ade6-L469*) as an intra- and interchromosomal recombination substrate (Osman *et al.*, 2000; Catlett and Forsburg, 2003). SCE is significantly elevated in $\Delta tel1$ and $\Delta cds1$ and mildly elevated in $\Delta chk1$ and $\Delta rad3$ mutants (Figure 4B), suggesting that some of the DNA damage incurred in $\Delta cds1$ mutants (and perhaps $\Delta chk1$ and $\Delta rad3$ as well) is repaired by sister chromatid recombination. As these increases in recombination occur without a substantial alteration in spore viability (Figure 4C) (Shimada *et al.*, 2002b), this result suggests that the levels of spontaneous damage we observe in checkpoint mutants are adequately repaired during the course of meiosis. Therefore, we suggest that loss of DNA checkpoint proteins during meiosis leads to an increase in spontaneous DNA damage which is adequately repaired by recombination by the time meiosis is complete. This repair occurs without slowing meiotic progression, further evidence that DNA damage does not trigger checkpoint-dependent arrest during fission yeast meiosis.

DISCUSSION

Fission yeast deficient for both *RAD54* homologues *rdh54+* and *rhp54+* progress through meiotic divisions in the presence of fragmented chromosomes (Catlett and Forsburg, 2003), raising the possibility that fission yeast cells do not arrest meiotic divisions in response to unrepaired or unrepairable DNA damage. Consistent with this possibility, we show that extensive DNA breaks, as marked by phospho-H2A domains, accumulate in $\Delta cds1$ and $\Delta rad3$ cells blocked in meiosis with HU (Figure 3), conditions under which these cells proceed with abnormal divisions (Murakami and Nurse, 1999) (Figure S1). Similarly, we show that treatments with the DNA damaging agents camptothecin and MMS, which result in increased abnormal chromosome segregation (Figure 1B), also fail to activate a checkpoint-dependent arrest of meiosis, as monitored by meiotic divisions and checkpoint protein mobility (Figure 1).

We also show that fission yeast assemble MI spindles in the presence of Rhp51 foci during a normal meiosis and with increasing frequency in the presence of elevated DNA damage (Figure 2), suggesting that fission yeast can proceed with at least the first meiotic division in the presence of recombination structures. Therefore, DNA damage from a variety of sources does not trigger checkpoint-mediated meiotic arrest in fission yeast, either in S-phase or later in meiotic prophase. This is in marked contrast to observations in budding yeast of checkpoint-dependent meiotic arrest in prophase (Lydall *et al.*, 1996) in response to incomplete recombination (Xu *et al.*, 1997), and may explain why some replication mutants in fission yeast do not cause meiotic arrest (Forsburg and Hodson, 2000).

It is not clear why DNA damage evades checkpoint activation during fission yeast meiosis. However, the absence of cross-over interference (Munz, 1994) and a classic synaptonemal complex (SC) (Bähler *et al.*, 1993) in fission yeast meiosis raises the possibility that checkpoint monitoring of damage repair and recombination may be dispensable during a simplified eukaryotic meiosis. Importantly, linear elements containing homologues of several of the proteins found in the SC do form during fission yeast meiosis (Lorenz *et al.*, 2004), indicating that conserved structural components are nevertheless required for efficient meiotic recombination (Molnar *et al.*, 2003).

Studies in budding yeast suggest that checkpoint proteins promote recombination in the context of the SC and chromosome cores, where cross-over events are favored and recombination can be monitored (Xu *et al.*, 1997; Grushcow *et al.*, 1999). As the SC and chromosome cores form during meiotic prophase (Roeder, 1997), damage early in S-phase might occur before the formation of meiotic chromosomal structures. Evidence of increased DSB formation in the *HIS4-LEU2* recombination hotspot in *Scrad24* and *Scrad17* mutants (Shinohara *et al.*, 2003), and chromosome fragmentation (Cha and Kleckner, 2002) and increased Rad52 focus formation (Lisby *et al.*, 2001) during S-phase in *Scmec1* mutants raises the possibility that loss of checkpoint function in *S. cerevisiae* could lead to increased DSB formation early in meiosis. We observe increased spontaneous DNA breaks during meiotic S-phase in fission yeast $\Delta cds1$ (and to a lesser extent, $\Delta rad3$) checkpoint mutants by phospho-H2A staining (Figure 3), demonstrating that loss of checkpoint proteins can lead to elevated spontaneous DNA

damage early in meiosis even in the absence of extraneous damage. This result suggests a possible nonessential role for checkpoint proteins during a normal meiotic S-phase.

If damage occurs before the formation of meiosis-specific chromosome structures, damage repair might show altered repair template and checkpoint surveillance characteristics. The increase in spontaneous DNA damage we observe in checkpoint mutants is repaired using both homologous chromosome and sister chromatid repair templates, as suggested by the partial rescue of $\Delta rec12$ spore viability, increased meiotic SCE, and high meiotic viability we observe in checkpoint mutants (Figure 4). Because S-phase damage from a variety of sources does not prevent chromosome segregation in MI, it is likely that the processing of spontaneous S-phase damage is not monitored by the recombination checkpoint; indeed, we see no evidence of meiotic delay in checkpoint mutants (Figure 1 and data not shown). Taken together, these data suggest that the partial $\Delta rec12$ rescue and elevated SCE we observe in some checkpoint mutants may be secondary to the repair of increased spontaneous S-phase damage.

Our data do not rule out a second, direct role for checkpoint proteins in the regulation of meiotic recombination, distinct from their role in preventing DNA damage in meiotic S-phase. Overall levels of meiotic gene conversion are modestly decreased in $cds1^+$ and $rad3^+$ checkpoint mutants (Shimada *et al.*, 2002b), suggestive of an additional, conserved role for checkpoint proteins in the regulation of meiotic recombination. The mammalian $rad3^+$ homolog ATR localizes to meiotic chromosome cores (Moens *et al.*, 1999; Tarsounas and Moens, 2001) while the *C. elegans* $cds1^+$ homologues Ce-CDS-1 and Ce-CDS-2 are required for meiotic crossing over (Oishi *et al.*, 2001), suggesting that roles in regulating meiotic recombination may be evolutionarily conserved. Our initial analysis of $\Delta tel1$ strains show an increase in SCE without a concomitant increase in $\Delta rec12$ rescue (Figure 4). This could mean that Tel1 is directly involved in the regulation of meiotic recombination without influencing levels of spontaneous damage in meiotic S-phase, in contrast to our results for Cds1 and Rad3. The prophase I defects (Barlow *et al.*, 1998) and infertility (Barlow *et al.*, 1996) observed in ATM deficient mice also reinforces the conserved importance of the Tel1 homolog in meiotic progression. Our observation of phospho-H2A domains in $\Delta rad3$ cells in meiotic S-phase suggests a role for Tel1 signaling early in meiosis; however a recent study in mammals showed that DNA-PK can also phosphorylate H2AX after IR (Stiff *et al.*, 2004). Future studies will determine the full extent of Tel1 functions during fission yeast meiosis.

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