Published in final edited form as:

Mutat Res. 2008 December 15; 648(1-2): 73–81. doi:10.1016/j.mrfmmm.2008.09.009.

UV but not X rays stimulate homologous recombination between sister chromatids and homologs in a *Saccharomyces cerevisiae* *mec1* (ATR) hypomorphic mutant

Michael Fasullo^{1,2,*} and Mingzeng Sun¹

¹ Ordway Research Institute, 150 New Scotland Avenue, Albany, New York 12208

² Department of Biomedical Sciences, School of Public Health, State University of New York, Albany, New York 12201

Abstract

MEC1, the essential yeast ATM/ATR homolog, prevents replication fork collapse and is required for the cellular response to DNA damage. We had previously observed higher rates of spontaneous SCE, heteroallelic recombination and translocations in *mec1-21* mutants, which still retain some G2 checkpoint function, compared to *mec1* null mutants, which are completely defective in checkpoint function, and wild type. However, the types of DNA lesions that are more recombinogenic in *mec1-21*, compared to wild type, are unknown. Here, we measured DNA damage-associated SCE, homolog (heteroallelic) recombination, and homology-directed translocations in *mec1-21*, and characterized types of DNA damage-associated chromosomal rearrangements that occur in *mec1-21*. Although frequencies of UV-associated recombination were higher in *mec1-21*, the mutant was defective in double-strand break-associated SCE and heteroallelic recombination. Over-expression of Rad53 in *mec1-21* reduced UV-associated recombination but did not suppress the defect in X-ray-associated recombination. Both X ray and UV exposure increased translocation frequencies in *mec1-21*, but the majority of the UV-associated products were non-reciprocal translocations. We suggest that although recombinational repair of double-strand breaks is less efficient in *mec1* mutants, recombinants may be generated by other mechanisms, such as break-induced replication.

Keywords

MEC1; homologous recombination; DNA repair; cell cycle checkpoint; yeast

1. Introduction

MEC1 is the essential yeast paralog [1] of the human ATM (mutated in ataxia telangiectasia, A-T) and the ortholog of ATR (ATM and *RAD3* related) (for review, see [2]). Similar to ATR, *MEC1* is required for both viability and for preventing replication fork collapse [3]. Both ATR and ATM hypomorphic alleles have been linked to higher incidence of cancer, which correlates

*Corresponding author and present address: *Ordway Research Institute, 150 New Scotland Avenue, Albany, New York 12208, Phone (518) 641-5457, Fax (518) 641-640, e-mail: E-mail: mfasullo@ordwayresearch.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

with a higher level of genetic instability present in ATR [4] and ATM [5] cell lines. How environmental agents, such as radiation and chemical carcinogens, increase genetic instability in ATR and ATM cell lines is not well understood. Since ATR null mutants are not viable, understanding DNA damage-associated genetic instability phenotypes of well defined yeast *mec1* hypomorphic mutants may thus provide insights into genetic instability phenotypes of ATR cells after exposure to DNA damaging agents.

mec1 mutants are hypersensitive to a wide variety of recombinogenic DNA damaging agents, such as X rays, UV, and MMS [6]. *MEC1* confers DNA damage resistance by activating downstream kinases, such as those encoded by *CHK1* and *RAD53* (Chk2), which, in turn, activate other effectors that trigger DNA damage-associated cell cycle arrest, maintain nucleotide balance, and repair double-strand breaks (DSBs). *MEC1* controls deoxynucleotide (dNTP) levels by activating Rad53, which in turn, activates the *DUN1*-mediated induction of ribonucleotide reductase [7]. *MEC1* is also required for the induction of Rad51 [8], and for the phosphorylation of Rad55 [9] and Sae2, a protein involved in processing DSBs [10]. Thus, the DNA damage sensitivity of *mec1* mutants may be due to deficiencies in multiple checkpoint functions.

mec1 hypomorphic mutants may exhibit different spontaneous hyper-recombination phenotypes than *mec1* null mutants. *mec1* null mutants exhibit high rates of spontaneous gross chromosomal rearrangements (GCRs, [11]) and loss of heterozygosity (LOH) [12] due to mitotic cross-overs, but only a modest increase in homologous recombination phenotypes. Alternatively, the hypomorphic missense mutant *mec1-21* [13], which is defective in S phase checkpoint function [14], but retains some G2 checkpoint function [15], exhibits a higher rates of spontaneous sister chromatid exchanges, gene conversion events, and ectopic recombination [16], but lower rates of spontaneous GCRs, compared to the *mec1* null [17]. The *mec1-21* mutant results from a G to A substitution (G882S) [13], which is outside the kinase domain but conserved with ATR. Although the differences in the recombination phenotypes could be due to multiple functions, one explanation is that DSBs can initiate mitotic, homologous recombination in some hypomorphic *mec1* mutants but not in the *mec1* null. Consistent with this explanation, higher frequencies of GCRs are observed in *mec1* null mutants after exposure to radiation or radiomimetic DNA damaging agents [18], while *mec1* mutants are deficient in MMS-associated heteroallelic events [9].

Since *mec1* null mutant contains secondary mutation(s) that increase dNTP levels, we asked which DNA damage-associated homologous recombination interactions are triggered in *mec1-21*, which is viable without secondary mutations. We had demonstrated that, compared to wild type, *mec1-21* exhibits higher rates of spontaneous, homologous recombination between sisters, homologs, and repeats on non-homologous chromosomes [16]. Here, we demonstrate that, compared to wild type, *mec1-21* exhibits higher frequencies of UV-associated but not X-ray-associated recombination between homologs and sister chromatids. Using a recombination assay that can detect non-reciprocal recombination, we found that the *mec1-21* diploid exhibits higher frequencies of UV-associated chromosomal non-reciprocal translocations, compared to wild type. Although we do not know all the mechanisms that stimulate homologous recombination in *mec1-21*, we suggest that one mechanism is break-induced replication (BIR).

2. Materials and methods

2.1. Media and yeast strains

Standard media for the culture of yeast, SC (synthetic complete, dextrose), SC-HIS (SC lacking histidine), SC-LEU (SC lacking leucine), SC-TRP (SC lacking tryptophan), SC-URA (SC lacking uracil), YP (yeast extract, peptone), and YPD (YP, dextrose), are described by Burke

et al. [19]. YPL medium contains YP with 2% lactate (pH 5.8); YPGlu medium contains YP medium with 2% ultra-pure glucose; YPGal medium contains YP medium with 2% ultra-pure galactose (Sigma, St Louis, MO). YP(A)D contains YPD with 80 mg/L adenine.

Relevant yeast strains are listed in Table 1. Haploid strains used to measure SCE contain two overlapping *his3* fragments [20], positioned in tandem at *trp1*, and were derived from YB163 [21]. The construction of the *mec1-21 rad52* strain was previously described [16]. Diploid strain (YB348) was used to measure translocations that were derived from a cross of one haploid (YB318) that contains the *his3* fragments on one copy of chromosomes II and IV [16,22], and another which did not contain the *his3* fragments (YB315). Heteroallelic recombination was measured by selecting for Ade⁺ recombinants between *ade2-a* and *ade2-n* [23,24] in the YB348 diploid strain. The *mec1-21* mutation was introduced in strains to measure frequencies of SCE, heteroallelic recombination, and translocations, as previously described [16].

To make the *mec1-Δ* mutant, we first introduced the *sml1::KanMX* or *sml1::URA3* allele [25] in the appropriate haploid strains, YB163, YB318, and YB318, by PCR-mediated gene replacement [26], and then introduced the *mec1-Δ::TRP1* or *mec1-Δ::KanMX* allele [25]. The primers used for amplifying *sml1::KanMX* or *sml1::URA3* were: 5' CATATCGTTACTGTTTGGAAACATCGC3' and 5' TAAAGGGAAAGGAAAATGCACG3'. The primers used for amplifying *mec1-Δ::TRP1* were 5'ATTCTTTTCAAGGCTCCATAACTA3' and 5' TTTTCCATATCTTCGAGCTCTTCTA3'.

2.3. Measuring rates of spontaneous recombination

The rates (events per cell division) of spontaneous, mitotic events that generate either SCE, heteroallelic recombination, or translocations were determined by the method of the median, [27] as executed by Esposito *et al.* [28], using 11 independent colonies for each rate calculation. For measuring rates of heteroallelic recombination, we obtained colonies from cells that were inoculated on YP(A)D medium, so that there was no growth selection for Ade⁺ recombinants. At least three independent rate calculations were done for each strain, and statistical significance was determined by the Mann-Whitney U-test [29].

2.4. Determining frequencies of DNA damage-associated recombinants

Protocols used to measure the recombinogenicity of UV and X-rays have been described [30]. At least three independent experiments were done for each DNA-damaging agent. We reported the spontaneous recombination frequencies [number of His⁺ recombinants per colony forming unit (CFU)], recombination frequencies obtained after exposure to DNA-damaging agents (stimulated frequency), and net frequencies for each DNA-damaging agent. The average net frequency of His⁺ recombinants was determined by first subtracting the spontaneous frequency from the stimulated frequency for each experiment and then taking the average.

2.5. Induction of HO endonuclease

pGHOT-*GAL3* [22], containing the HO gene under *GAL* control, was introduced into *MEC1*, *mec1-21* and *mec1-Δ* strains by selecting for Trp⁺ transformants. After growth in SC-TRP medium, cells were diluted 1:10 in YPLactate and incubated for a minimum of 12 hr. At a density of 10⁷ cells/ml, glucose or galactose was added to a final concentration of 2%, to either repress or induce the expression of HO endonuclease, respectively. After 2 hr, cells were plated directly on YPD medium for viability and on SC-HIS to measure recombination. Colonies appearing on YPD medium were replica plated on SC-TRP to measure the number of Trp⁺ colonies containing the pGHOT-*GAL3* plasmid.

2.6. Verification that His⁺ recombinants result from unequal SCE or intrachromatid recombination

Mitotic unequal SCE between *his3-Δ5'* and *his3-Δ3'* results in His⁺ recombinants that contain *HIS3* flanked by *his3-Δ5'* and *his3-Δ3'* (Figure 1; 21). Three different mechanisms may participate in the repair of the DSB generated by HO endonuclease digestion at the *trp1::his3-Δ3':HOcs* locus [31]. These include NHEJ, single-strand annealing (SSA), and homologous recombination. SSA events generate a chromosomal deletion and a single *his3* fragment lacking both 3' and 5' ends, rendering cells unable to generate His⁺ recombinants (Figure 1). To determine frequencies of chromosomal deletions (SSA), cells were plated on YPD plates after HO induction. The surviving colonies were replica plated onto SC-HIS to measure the number of His⁺ colonies that cannot generate His⁺ recombinants and onto SC-TRP to measure the number of colonies that maintain the pGHOT-GAL3 plasmid. The presence of the single *his3* fragment was confirmed by the presence of a 1.4-Kb PCR product using primers 5'-CACGGCAGAGACCAATCAGTA-3' and 5'-GCACTCCTGATTCCGCTAATA-3' [31].

2.7. Chromosomal DNA gels

Undigested yeast chromosomal DNA was resolved on contour-clamped homogeneous electric field (CHEF) gels containing 1% agarose [32]. The gels were run at 220 V (6 V/cm) for 26 hr at a 90-sec pulse time [22]. Chromosomal DNA was transferred to nylon after exposure to 60 J/m² of UV radiation for Southern blot analysis [22,33]. The 1.7-kb *Bam*HI *HIS3* fragment was used as a probe.

3. Results

We hypothesized that *mec1* mutants defective in the S phase checkpoint could potentially exhibit hyper-recombination phenotypes due to collapsed replication forks or the accumulation of single-stranded DNA. We measured DNA damage-associated recombination in the *mec1-21* [14] mutant, which is still proficient at triggering G2 arrest after radiation exposure [15]. The recombination assays are shown in Figure 1. Unequal SCE was measured by selecting for His⁺ recombinants in haploid strains containing two truncated *his3* gene fragments [21]. Diploid strains were used to measure heteroallelic recombination between *ade2-a* and *ade2-n* [24] and ectopic recombination between *GAL1::his3-Δ5'* and *trp1::his3-Δ3'* [22].

3.1. Exposure to UV but not X rays stimulates more SCE in *mec1-21* mutants than in wild type

We hypothesized that the observed hyper-recombination phenotype resulted from repair of DNA lesions generated in S phase. We therefore expected that higher DNA damage-associated SCE would occur in *mec1-21*, compared to wild type, in the presence of DNA adducts that block or hinder replication. We measured frequencies of UV-associated SCE in *mec1-21* and in wild type (Figure 2). After log phase cells were exposed to UV, we observed eight-fold increased SCE frequencies in wild type, consistent with previous observations [22,31], but much higher frequencies in *mec1-21*. After exposure to 90 J/m², the net UV-associated SCE frequency was 2×10^{-4} in *mec1-21*, ~fourfold higher than in wild type; net frequencies of UV-associated SCE did not increase at higher doses of UV. Thus, *mec1-21* exhibits higher frequencies of UV-associated SCE, compared to wild type.

We also measured the effect of X-ray exposure, and observed that SCE frequencies increased fourfold in wild type after X-ray exposure, but less than twofold in *mec1-21* and *mec1-Δ* mutants (Figure 2). Although wild-type cells in liquid medium appear as X-ray sensitive as *mec1-21* cells, we observed that *mec1-21* cells were ~50% more X-ray sensitive after being plated and then irradiated [15]. We observed a similar deficiency in X-ray-associated SCE in a *mec1-21* diploid mutant (data not shown).

3.2. *mec1-21* is deficient in the stimulation of unequal SCE by HO-induced double-strand breaks

Since X-rays generate both SSBs and DSBs, we determined whether unequal SCE frequencies increased after a single HO-induced DSB was targeted to *his3-Δ3'::HOcs* in *mec1* mutants (Table 2). We introduced pGHOT-GAL3, containing the galactose-inducible *HO* gene, into wild type and *mec1* mutants in which the *MAT* locus (*MATa-inc*) is not a substrate for HO endonuclease. At *trp1::his3-Δ3'::HOcs*, the HO-induced DSB can be repaired by SSA or NHEJ mechanisms (Figure 1). After induction of *HO* in log phase cells, the reduced viability in wild type was similar to what has been previously observed [22,31]. Viability was reduced over 50% reduction in *mec1-Δ* cells, consistent with this mutant deficiency in SSA (34). This was supported by observations that unselected *mec1-Δ* colonies arising after *HO* induction contain fewer deletions (26%, 35/129), compared to wild type (72%, 36/50). After *HO* induction, SCE frequencies were increased 10-fold in wild-type and *sml1* cells, but only threefold in *mec1-21* and twofold in *mec1-Δ*. After *HO* induction, SCE frequencies were increased 10-fold in wild type but only threefold in *mec1-21*. Thus, DSB-associated unequal SCE is *MEC1*-dependent.

3.3. Suppression of spontaneous and UV-associated SCE hyper-recombination by RAD53 overexpression

Activation of Rad53 is *MEC1*-dependent, and *RAD53* over-expression has been previously shown to suppress the HU and UV sensitivity phenotypes of *mec1-21* (7). We determined whether *RAD53* over-expression would also suppress the recombination phenotypes of *mec1-21*(YB311). We introduced the *RAD53* gene on pWL25 [35], containing *URA3*. Ura⁺ transformants were grown in SC-URA medium and Rad53 overproduction was confirmed by Western blots (supplemental Figure 1). Rates of spontaneous SCE in *mec1-21* + pWL25 (YB376) were reduced but still threefold greater than wild type (Table 3). Rates of spontaneous SCE in *mec1-21* containing an empty vector (YCp50) were similar than those of *mec1-21*. Net frequencies of UV-associated SCE were measured after exposure to 90 J/m². In *mec1-21*, *mec1-21* + pWL25, and wild type, the net frequencies were $(204 \pm 56) \times 10^{-6}$, $(38 \pm 6) \times 10^{-6}$, $(36 \pm 11) \times 10^{-6}$, respectively. However, frequencies of X-ray associated SCE did not increase in *mec1-21* + pWL25 but did increase sevenfold in *MEC1* (YB163) + pWL25 after exposure to 6 krad X rays. Thus, *RAD53* over-expression in *mec1-21* reduces spontaneous and UV-associated SCE, but does not significantly increase X-ray-associated SCE.

Similarly, *mec1-21* cells containing a galactose-inducible *RAD53* gene on pJA98, also exhibited lower frequencies of recombination after Rad53 was overproduced. Because the *mec1-21* strain contains *trp1-Δ1* and lacks the *GAL3* promoter, we first introduced pGAL3 (*TRP1*) and later pJA98 (*URA3*) [7]. Approximately 10⁶ cells, obtained from independent Ura⁺ Trp⁺ transformants, were inoculated in SC (glucose) -URA-TRP and in SC (galactose) -URA-TRP, and cultures were incubated for 4–5 generations. Recombination frequencies ($2.7 \pm 0.4 \times 10^{-5}$) were twofold lower ($P < 0.01$, $n = 11$) from cells grown in galactose medium, compared with cells grown in glucose medium ($6.8 \pm 1.4 \times 10^{-5}$, $n = 11$). There was no difference ($P = 0.5$) in recombination frequencies between *mec1-21* + pGAL3 cells grown in glucose ($8.7 \pm 0.4 \times 10^{-5}$) or in galactose ($9 \pm 0.6 \times 10^{-5}$). Thus, inducible *RAD53* expression decreases recombination in *mec1-21*.

3.4. *mec1-21* diploid mutants exhibit UV-associated homolog recombination but are deficient in X-ray-associated homolog recombination

To determine whether homologs were better substrates for recombinational repair than sister chromatids in *mec1* mutants, we measured UV and X-ray associated heteroallelic recombination. X-ray exposure increased the frequencies of heteroallelic recombination in *mec1-21* and wild type by two-fold ($P < 0.05$) and 30-fold, respectively (Figure 3). However,

UV-associated frequencies of heteroallelic recombination were as high in *mec1-21* as in wild type (Figure 3). The *mec1-21* diploid is thus deficient in X-ray-associated but not in UV-associated homolog recombination.

3.5. Higher frequencies of radiation-associated translocations occur in *mec1-21* compared to wild type

If DSB repair is less efficient between homologs and sister chromatids, we asked whether *mec1* mutants exhibit more ectopic recombination between repeated sequences on non-homologs. We selected for His⁺ recombinants in which mitotic recombination between *his3* fragments on chromosomes II and IV generates reciprocal translocations. We previously observed a 23-fold increase in spontaneous translocations in *mec1-21*. Here, we observed higher frequencies of radiation-associated translocations in *mec1-21* diploids, compared to wild type (Figure 3). Net frequencies of X-ray associated translocations were between twofold and threefold higher in *mec1-21* mutant than in wild type, while net frequencies of UV-associated translocations were between sixfold and 50-fold higher than in wild type. Thus, frequencies of radiation-associated translocations are higher in *mec1-21*, compared to wild type.

3.6. Electrophoretic karyotypes of chromosome rearrangements from *mec1-21* and *mec1-Δ* mutants

We previously observed that, compared to wild type, the *rad9* mutant [22], defective in G2 checkpoint, and the *rad51* diploid mutant [21], defective in recombinational repair, exhibit high frequencies of spontaneous and radiation-associated translocations. In diploids, viable His⁺ recombinants can be selected that contain *CEN2::IV* but lack the *CEN4::II* translocated chromosome, since *CEN4::II* does not contain *HIS3* (Figure 1). Recombination events that do not generate both reciprocal translocations are referred to as non-reciprocal events. Since, we observed that many recombinants in G2 checkpoint and recombination mutants contain non-reciprocal translocations [21,22], we compared the electrophoretic karyotypes of spontaneous and UV-associated His⁺ recombinants from *mec1-21*, *mec1-Δ*, and wild type (Figure 4). In wild type, most independent, spontaneous and UV-associated His⁺ recombinants contained reciprocal translocations (Table 4). In the *mec1-21* (YB325) strain, the majority (5/8) of the UV-associated His⁺ recombinants contained non-reciprocal translocations, but non-reciprocal translocations (1/8) were more difficult to detect among spontaneous recombinants. His⁺ recombinants that contain non-reciprocal translocations typically contain *his3* sequences on chromosome II [20]. By CHEF, we determined that in *mec1-21*, 25% (4/16) of the His⁺ recombinants contained other rearrangements, some of which hybridize to *his3* sequences. The majority (6/8) of spontaneous His⁺ recombinants in the *mec1-Δ* mutant contained non-reciprocal translocations, while UV-associated recombinants contained both non-reciprocal and reciprocal rearrangements, and about half (7/16) of the His⁺ recombinants from the *mec1-Δ* mutant contained other rearrangements (Figure 4). Thus, non-reciprocal translocations occurred in both *mec1-21* and in *mec1-Δ*, but were seldom observed in wild type.

3.7. Growth phenotypes and UV sensitivities of *mec1-21* and *mec1-21 rad52* mutants

One explanation for the hyper-recombination in *mec1-21*, is that more DSBs are spontaneously generated or occur after UV exposure in *mec1-21*. We had previously observed a synergistic increase in the UV sensitivity of *mec1-21 rad52* double mutant, compared to the single mutants [16]. Compared to wild type (YB163), which has a doubling time of 1.9 hrs and a plating efficiency of 91%, the *mec1-21*(YB312) and *rad52* (YB328) mutants exhibited modest increases in doubling time and decreases in plating efficiency (Table 5). The plating efficiency of *mec1-21 rad52* (YB347) mutant was less than half that of wild type, and the doubling time of *mec1-21 rad52* was twofold higher than *mec1-21* and significantly higher than *rad52* ($P <$

0.05). These data suggest that more DSBs spontaneously occur in *mec1-21*, compared to wild type, and that *RAD52* confers UV resistance in *mec1-21*.

4. Discussion

The ATR (ATM) yeast homolog *MEC1* plays a pivotal role in sensing and transmitting DNA damage signals. Because both ATR and *MEC1* are essential [1,2], understanding the genetic instability phenotypes in *mec1* hypomorphic mutants may yield insights into genetic instability of cells defective in ATR. We had previously observed that *mec1-21*, a missense mutant [13], exhibits higher frequencies of spontaneous homologous recombination, and that the hyper-recombination requires *RAD9*, *CHK1*, and *PDS1* [16]. However, it was unknown whether exposure to recombinogenic DNA damaging agents would further increase frequencies of homologous recombination. Here, we observed higher frequencies of SCE and heteroallelic recombination in *mec1-21* after exposure to UV but not X rays, compared to wild type. Higher frequencies of UV-associated non-reciprocal translocations suggest that in *mec1-21* a possible mechanism for DNA damage-associated recombination is BIR. The high frequencies of UV-associated SCE can be partially suppressed by over-expression of Rad53, suggesting that one *MEC1* function in suppressing UV-associated recombination is Rad53 signaling. These studies indicate that genetic instability in *mec1* (ATR) cells depends on the specific DNA damaging agent.

These conclusions were based on the *mec1-21* missense mutation, which does not confer the same G2 checkpoint deficiencies as *mec1-Δ* [15]. We do not know the kinase activity and substrate specificity of the Mec1 protein encoded by *mec1-21*. Further studies are necessary to determine whether *TEL1* is required for homologous recombination phenotypes in *mec1* hypomorphic mutants.

4.1. Comparison with other checkpoint and rad mutants

Other DNA repair and checkpoint mutants also exhibit UV-associated recombination but not X-ray-associated recombination between sister chromatids. For example, both *rad9* [22] and *rad53* [36] exhibit UV-associated recombination but do not exhibit X-ray associated recombination between sister chromatids. *RAD9* is required for multiple functions in yeast, including radiation-associated G2 arrest [37], and transcriptional induction of DNA repair genes after radiation exposure [38], and the tolerance of irreparable UV-induced DNA damage [39]. *RAD9*-mediated functions are partially conferred by activating Rad53, which, in turn, activates Dun1 and triggers the DNA damage-inducibility of ribonucleotide reductase [40, 41]. Considering that radiation exposure still triggers G2 arrest but poorly activates Rad53 in *mec1-21* [15], our data suggest that Rad53 activation may be important for recombinational repair of DSBs, but dispensable for stimulating recombination after exposure to UV. Exactly which downstream effectors of Rad53 are important in X-ray-associated homologous recombination are not known, since *dun1* mutants, defective in the induction of *RNR*, still exhibits X-ray-associated SCE and homolog recombination [42].

Particular *rad* mutants, defective in DSB repair, share some homologous recombination phenotypes with *mec1-21*. For example, *rad52-c* mutants are defective in X-ray-associated heteroallelic recombination but are still proficient at UV-associated recombination [43]. UV-associated recombination has also been observed in *rad52* null mutants, but the recombination frequencies are much lower than wild type [44]. *rad51* mutants, although defective in all types of DNA damage-associated SCE, still exhibits UV-associated non-reciprocal translocations that result from homologous recombination [21]. Thus, *rad* mutants defective in recombinational repair of DSBs may still exhibit UV-associated homologous recombination. Further genetic studies are necessary to determine whether *rad52-c* and *mec1-21* mutants are defective in the same pathways for UV and DSB repair.

4.2. Higher levels of DNA damage-associated recombination observed in *mec1-21* may be initiated by ssDNA or occur by BIR

Although the well known pathway for initiating homologous recombination is the DSB pathway (gap repair) described by Szostak *et al.* [45], we observed that both HO-induced DSBs and X rays were less efficient in stimulating recombination in *mec1-21*, compared to wild type. It is thus unlikely that repair of DSBs by gap repair can account for all the hyper-recombination observed in *mec1-21*. We suggest two possible recombination mechanisms that explain why UV but not X ray exposure stimulates recombination in *mec1-21*. First, DSBs may initiate BIR, which could then generate recombinants. Second, recombinational repair may be initiated by single-stranded DNA, which may accumulate due to incomplete DNA replication.

BIR could occur when a replication fork collapses, thus yielding a DSB. Restoration of the DNA replication fork occurs after strand invasion of the broken chromatid with the incompletely replicated sister chromatid. Using the unbroken chromatid as a template, the recombination intermediate can thus prime DNA synthesis and replication could continue to the telomere of the unbroken chromatid [46]. Our data supports the idea that the broken chromatid could serve as a substrate for strand invasion of a repeated sequence on a non-homologous chromosome, which would generate non-reciprocal translocations. The notion that DSBs arise spontaneously in *mec1-21* is supported by the observation that the *mec1-21 rad52* mutant exhibits a very low plating efficiency. We speculate that *mec1* deficiency in signaling Rad53 may contribute to spontaneous or UV-induced replication fork collapse, since *RAD53* is required to stabilize replication forks after exposure to DNA damaging agents [47].

However, we cannot exclude the possibility that ssDNA initiates homologous recombination in *mec1-21*. This possibility is supported by observations that ssDNA accumulates in other *mec1* hypomorphs, such as *mec1-srf* [48], and that ssDNA is postulated to initiate recombination in other DNA repair mutants, such as in *sgs1* [49] and in *rad52-C* [43]. *SGS1* and *MEC1* have been previously suggested to participate in different pathways for stabilizing replication forks [50]. Further studies are necessary to determine whether ssDNA spontaneously accumulate in *mec1-21* or during the course of UV excision repair.

In summary, *mec1-21* exhibits both spontaneous and UV-associated hyper-recombination. Since DSB repair mechanisms mediated by homologous recombination are less efficient in *mec1-21*, recombination initiated by BIR or ssDNA may explain some of the hyper-recombination phenotypes. It would thus be interesting to know whether *SGS1* and *MEC1* participate different pathways for suppressing homologous recombination in response to DNA damaging agents. These studies demonstrate that *mec1* hypomorphic mutants that retain some G2 checkpoint functions can still exhibit significant hyper-recombination phenotypes that depend on the DNA damaging agent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by grant CA70105 from the National Cancer Institute and a grant No. 1-FY01-629 from the March of Dimes. We thank Tom Petes for *mec1-21*, R. Rothstein for *mec1* and *sml1* deletion strains. We thank C. Cera for carefully reading this manuscript.

References

1. Kato R, Ogawa H. An essential gene, *ESR1*, is required for mitotic cell growth, DNA repair and meiotic recombination in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 1994;22:3104–3112. [PubMed: 8065923]
2. Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nature Reviews* 2003;3:155–168.
3. Cha RS, Kleckner N. ATR homolog Mec1 promotes fork progression, thus averting breaks in replication slow zones. *Science* 2002;297:602–606. [PubMed: 12142538]
4. Syrou M, Gergioui I, Paschopoulos M, Lolis D. Seckel syndrome in a family with three affected children and hematological manifestations associated with chromosome instability. *Genet Couns* 1995;6:37–41. [PubMed: 7794560]
5. Mavrou A, Tsangaris GT, Roma E, Kolialexi A. The ATM gene and ataxia telangiectasia. *Anticancer Res* 2008;28:401–415. [PubMed: 18383876]
6. Morrow DM, Tagle DA, Shiloh Y, Collins FS, Hieter P. TEL1, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene *MEC1*. *Cell* 1995;82:831–840. [PubMed: 7545545]
7. Sanchez Y, Desany BA, Jones WJ, Liu Q, Wang B, Elledge SJ. Regulation of *RAD53* by the ATM-like kinases *MEC1* and *TEL1* in yeast cell cycle checkpoint pathways. *Science* 1996;271:357–360. [PubMed: 8553072]
8. Gasch AP, Huang M, Metzner S, Botstein D, Elledge SJ, Brown PO. Genomic expression responses to DNA-damaging agents and the regulatory role of the yeast ATR homolog Mec1p. *Mol Biol Cell* 2001;12:2987–3003. [PubMed: 11598186]
9. Bashkurov VI, King JS, Bashkurova EV, Schmuckli-Maurer J, Heyer WD. DNA repair protein Rad55 is a terminal substrate of the DNA damage checkpoints. *Mol Cell Biol* 2000;20:4393–4404. [PubMed: 10825202]
10. Baroni E, Viscardi V, Cartagena-Lirola H, Lucchini G, Longhese MP. The functions of budding yeast Sae2 in the DNA damage response require Mec1- and Tel1-dependent phosphorylation. *Mol Cell Biol* 2004;24:4151–4165. [PubMed: 15121837]
11. Myung K, Chen C, Kolodner RD. Multiple pathways cooperate in the suppression of genome instability in *Saccharomyces cerevisiae*. *Nature* 2001;411:1073–1076. [PubMed: 11429610]
12. Craven R, Greenwell P, Dominska M, Petes T. Regulation of genome stability by *TEL1* and *MEC1*, yeast homologs of the mammalian ATM and ATR genes. *Genetics* 2002;161:493–507. [PubMed: 12072449]
13. Mallory JC, Petes TD. Protein kinase activity of Tel1p and Mec1p, two *Saccharomyces cerevisiae* proteins related to the human ATM protein kinase. *Proc Natl Acad Sci U S A* 2000;97:13749–13754. [PubMed: 11095737]
14. Desany BA, Alcibasas AA, Bachant JB, Elledge SJ. Recovery from DNA replicational stress is the essential function of the S-phase checkpoint pathway. *Genes Dev* 1998;12:2956–2970. [PubMed: 9744871]
15. Sun M, Fasullo M. Activation of the budding yeast securing Pds1 but not Rad53 correlates with double-strand break-associated G2/M cell cycle arrest in a *mec1* hypomorphic mutant. *Cell Cycle* 2007;6:1896–1902. [PubMed: 17671432]
16. Fasullo M, Sun M. The *Saccharomyces cerevisiae* checkpoint genes *RAD9*, *CHK1*, and *PDS1* are required for elevated homologous recombination in a *mec1* (ATR) hypomorphic mutant. *Cell Cycle* 2008;7:1–9.
17. Craven R, Greenwell P, Dominska M, Petes T. Regulation of genome stability by *TEL1* and *MEC1* yeast homologs of the mammalian ATM and ATR genes. *Genetics* 2002;161:493–507. [PubMed: 12072449]
18. Myung K, Kolodner RD. Induction of genome instability by DNA damage in *Saccharomyces cerevisiae*. *DNA Repair* 2003;2:243–258. [PubMed: 12547388]
19. Burke, D.; Dawson, D.; Stearns, T. A Cold Spring Harbor Laboratory Course Manual. Cold Spring Harbor Press; New York: Methods in yeast genetics.

20. Fasullo MT, Davis RW. Recombination substrates designed to study recombination between unique and repetitive sequences in vivo. *Proc Natl Acad Sci USA* 1987;84:6215–6219. [PubMed: 3306671]
21. Fasullo MT, Giallanza P, Bennett T, Cera C, Dong Z. *Saccharomyces cerevisiae rad51* mutants are defective in DNA damage-stimulated sister chromatid exchange but exhibit increased rates of homology-directed translocations. *Genetics* 2001;158:959–972. [PubMed: 11454747]
22. Fasullo MT, Bennett T, AhChing P, Koudelik J. The *Saccharomyces cerevisiae RAD9* checkpoint reduces the DNA damage-associated stimulation of directed reciprocal translocations. *Mol Cell Biol* 1998;18:1190–2000. [PubMed: 9488434]
23. Huang KN, Symington LS. Mutation of the gene encoding protein kinase C 1 stimulates mitotic recombination in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1994;14:6039–6045. [PubMed: 8065337]
24. Bai Y, Symington LS. A Rad52 homolog is required for *RAD51*-independent mitotic recombination in *Saccharomyces cerevisiae*. *Genes Dev* 1996;10:2025–2037. [PubMed: 8769646]
25. Zhao X, Georgieva B, Chabes A, Domkin V, Ippel JH, Schleucher J, Wijmenga S, Thelander L, Rothstein R. Mutational and structural analyses of the ribonucleotide reductase inhibitor Sml1 define its Rnr1 interaction domain whose inactivation allows suppression of *mec1* and *rad53* lethality. *Mol Cell Biol* 2000;20:9076–9083. [PubMed: 11074005]
26. Rothstein RJ. One-step gene disruption in yeast. *Methods Enzymol* 1983;101:202–211. [PubMed: 6310324]
27. Lea DE, Coulson CA. The distribution of the numbers of mutants in bacterial populations. *J Genet* 1949;49:264–284.
28. Esposito MS, Maleas DT, Bjornstad KA, Bruschi CV. Simultaneous detection of changes in chromosome number, gene conversion and intergenic recombination during mitosis of *Saccharomyces cerevisiae*. *Curr Genet* 1982;6:5–11.
29. Zar, JH. *Biostatistical Analysis*. Prentice Hall; Englewood Cliffs, N. J: 1996.
30. Fasullo MT, Dave P. Mating type regulates the radiation-associated stimulation of reciprocal events in *Saccharomyces cerevisiae*. *Mol Gen Genet* 1994;243:63–70. [PubMed: 8190072]
31. Dong Z, Fasullo M. Multiple recombination pathways for spontaneous and DNA damage-associated sister chromatid exchange in *Saccharomyces cerevisiae*: Role of *RAD1* and the *RAD52* epistasis group genes. *Nucleic Acids Res* 2003;31:2576–2585. [PubMed: 12736307]
32. Chu GD, Vollrath D, Davis RW. Separation of large DNA molecules by contour-clamped homogeneous electric fields. *Science* 1986;234:1582–1585. [PubMed: 3538420]
33. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975;98:503–517. [PubMed: 1195397]
34. Kaye JA, Melo JA, Cheung SK, Vaze MB, Haber JE, Toczyski DP. Genomic instability by impeding proper chromosome segregation. *Curr Biol* 2004;14:2096–2106. [PubMed: 15589151]
35. Kim S, Weinert TA. Characterization of the checkpoint gene *RAD53/MEC2* in *Saccharomyces cerevisiae*. *Yeast* 1997;13:735–745. [PubMed: 9219338]
36. Fasullo M, Sun M, Dong Z. *Saccharomyces cerevisiae RAD53* (CHK2) but not *CHK1* is required for double-strand break-initiated SCE and DNA damage-associated SCE after exposure to X rays and chemical agents. *DNA Repair* 2003;4:1240–1251. [PubMed: 16039914]
37. Weinert TA, Hartwell LH. Characterization of the *RAD9* gene of *Saccharomyces cerevisiae* and evidence that it acts posttranslationally in cell cycle arrest after DNA damage. *Mol Cell Biol* 1990;10:6554–6564. [PubMed: 2247073]
38. Aboussekhra A, Vialard JE, Morrison DE, de la Torre-Ruiz MA, Cernáková L, Fabre F, Lowndes NF. A novel role for the budding yeast *RAD9* checkpoint gene in DNA damage-dependent transcription. *EMBO J* 1996;15:3912–3922. [PubMed: 8670896]
39. Paulovich AG, Armour CD, Hartwell LH. The *Saccharomyces cerevisiae RAD9*, *RAD17*, *RAD24* and *MEC3* genes are required for tolerating irreparable, ultraviolet-induced DNA damage. *Genetics* 1999;150:75–93. [PubMed: 9725831]
40. Navas TA, Sanchez Y, Elledge SJ. *RAD9* and DNA polymerase epsilon form parallel sensory branches for transducing the DNA damage checkpoint signal in *Saccharomyces cerevisiae*. *Genes Dev* 1996;10:2632–2643. [PubMed: 8895664]

41. Vialard JE, Gilbert CS, Green CM, Lowndes NF. The budding yeast Rad9 checkpoint protein is subjected to Mec1/Tel1-dependent hyperphosphorylation and interacts with Rad53 after DNA damage. *EMBO J* 1998;17:5679–5688. [PubMed: 9755168]
42. Fasullo M, Koudelik J, AhChing P, Giallanza P, Cera C. Radiosensitive and mitotic recombination phenotypes of the *Saccharomyces cerevisiae dun1* mutant defective in DNA damage-inducible gene expression. *Genetics* 1999;152:909–919. [PubMed: 10388811]
43. Lettier G, Feng Q, de Mayolo AA, Erdeniz N, Reid RJ, Lisby M, Mortensen UH, Rothstein R. The role of DNA double-strand breaks in spontaneous homologous recombination in *S. cerevisiae*, *PLoS Genet* 2006;2:e194. [PubMed: 17096599]
44. Coïc E, Feldman T, Landman AS, Haber JE. Mechanisms of Rad52-independent spontaneous and UV-induced mitotic recombination in *Saccharomyces cerevisiae*. *Genetics* 2008;179:199–211. [PubMed: 18458103]
45. Szostak JW, Orr-Weaver TL, Rothstein RJ, Stahl FW. The double-strand-break repair model for recombination. *Cell* 1983;33:25–35. [PubMed: 6380756]
46. Kraus E, Leung WY, Haber JE. Break-induced replication: a review and an example in budding yeast. *Proc Natl Acad Sci U S A* 2001;98:8255–8262. [PubMed: 11459961]
47. Segurado M, Diffley JF. Separate roles for the DNA damage checkpoint protein kinases in stabilizing DNA replication forks. *Genes Dev* 2008;22:1816–1827. [PubMed: 18593882]
48. Merrill BJ, Holm C. A requirement for recombinational repair in *Saccharomyces cerevisiae* is caused by DNA replication defects of *mec1* mutants. *Genetics* 1999;153:595–605. [PubMed: 10511542]
49. Fabre F, Chan A, Heyer WD, Gangloff S. Alternate pathways involving Sgs1/Top3, Mus81/Mms4, and Srs2 prevent formation of toxic recombination intermediates from single-stranded gaps created by DNA replication. *Proc Natl Acad Sci U S A* 2002;99:16887–16892. [PubMed: 12475932]
50. Cobb JA, Schleker T, Rojas V, Bjergbaek L, Tercero JA, Gasser SM. Replisome instability, fork collapse, and gross chromosomal rearrangements arise synergistically from Mec1 kinase and RecQ helicase mutations. *Genes Dev* 2005;19:3055–3069. [PubMed: 16357221]

Figure 1.

SCE, translocation, and heteroallelic recombination assays used in this study. Ovals represent centromeres and lines represent chromosomes. For simplicity, the left arms of chromosomes are not included. An arrow and feathers together denote *HIS3*. As indicated in the bottom left of the figure, the 5' deletion lacks the feather and the 3' deletion lacks the arrow. The two regions of the sequence identity shared by the *his3* fragments are indicated by decorated boxes; closely-spaced diagonal-filled boxes indicate a region of 167 bp, and the broadly-spaced diagonal line-filled boxes indicate a region of ~300bp. The 117-bp HO cut site (*HOcs*), as indicated by an arrowhead, is located between these sequences within the *his3-Δ3'::HOcs* fragment. The "X" indicates where recombination occurred. The *his3*-truncated fragments are integrated into the *trp1* locus to measure SCE events. His⁺ recombinants resulting from SCE were selected after exposure to radiation or after the induction of HO endonuclease (A, left). Right panel of the figure indicates that HO endonuclease-induced DSB can be repaired by the SSA pathway, which generates intrachromosomal deletions. The deletion renders cells unable to generate His⁺ recombinants, and can be detected by PCR using primers #1 5' CACGGCAGAGACCAATCAGTA3' and #2 5'GCACTCCTGATTCGCTAATA3'. (B) Translocation events result from recombination between the same *his3* fragments located each

on chromosomes II and IV. Positions of the *GAL1* and *trp1* are shown on chromosomes II, IV, and the reciprocal translocation. The translocated chromosome that contains *CEN2* linked to *HIS3* and the long arm of chromosome IV is referred to as *CEN2::IV*, while the translocated chromosome that contains *CEN4* linked to the long arm of chromosome II is referred to as *CEN4::II*. Diploid strains contain one copy of chromosome II and IV that do not contain *his3* sequences. (C) Heteroallelic recombination between *ade2-a* and *ade2-n* generates *ADE2*. *ADE2* and *ade2* alleles are represented as boxes; *ade2-a* and *ade2-n* are separated by approximately 1 kb.

Figure 2.

SCE frequencies in wild type (*MEC1*), *mec1-Δ* and *mec1-21* strains after UV and X-ray exposure. Strains used include *MEC1*(diamond, YB163), *mec1-21* (triangle, YB311) and *mec1-ΔsmI1*(square, YB327). SCE was measured by selecting for His⁺ recombinants. Log phase cells were exposed to radiation and then plated on SC-HIS to measure recombination and the appropriate dilution was plated on YPD to measure viability. Percent survival is the number of CFU after radiation/number of CFU before radiation × 100%. Top panels show data after cells were exposed to UV and bottom panels show data after cells were exposed to X rays. (A) Percent survival after cells were exposed to UV. (B) Frequency of His⁺ recombinants (number of His⁺ recombinants/c.f.u.) plotted against UV dose. (C) Net frequency of His⁺ recombinants (UV-associated frequency – spontaneous frequency) plotted against UV dose. (D) Percent survival after cells were exposed to X rays. (E) Frequency of His⁺ recombinants (number of His⁺ recombinants/c.f.u.) plotted against X-ray dose. (F) Net Frequency of His⁺ recombinants (X-ray-associated frequency – spontaneous frequency) plotted against X ray dose. 10 Gy = 1 krad.

Figure 3.

DNA damage associated frequencies of homology-directed translocations and heteroallelic recombination in *MEC1* (black diamond, YB348), *mec1-21* (black triangle, YB325), and *mec1 sml1* (black square, YB324) diploid strains. To measure translocation frequency, we selected for His⁺ recombinants and to measure heteroallelic recombination, we selected for Ade⁺ recombinants. Top panels show data after cells were exposed to X rays and bottom panels show data after cells were exposed to UV. Percent survival is the number of CFU after radiation/ number of CFU before radiation $\times$ 100%. (A) Percent survival after cells were exposed to X rays. (B) Frequency of His⁺ recombinants (number of His⁺ recombinants/c.f.u.) plotted against X-ray dose. (C) Frequency of Ade⁺ recombinants (number of Ade⁺ recombinants/c.f.u.) plotted against X-ray dose. (D) Percent survival after cells were exposed to UV. (E) Frequency of His⁺ recombinants (translocations) (number of His⁺ recombinants/c.f.u.) plotted against UV dose. (F) Frequency of Ade⁺ recombinants (heteroallelic recombination) (number of Ade⁺ recombinants/c.f.u.) plotted against UV dose.

Figure 4.

Electrophoretic karyotype of spontaneous and DNA damage-associated His⁺ recombinants resulting from ectopic recombination between *GAL1::his3-Δ5'* and *trp1::his3-Δ 3'* in the *mec1-21* (YB325) and *mec1-Δ* (YB324) diploids. (Left) Southern blot using the ³²P-labeled 1.7-kb *Bam*HI *HIS3* fragment. (Right) Picture of the ethidium-bromide-stained gel. Arrows point to the well, wild-type chromosomes II and IV, and the translocations *CEN2::IV* and *CEN4::II*. Hybridization to chromosome XV results from the DNA homology between the *his3-Δ 200* locus and the sequences that flank *HIS3* within the 1.7-kb *Bam*HI fragment. Lanes: (A) His⁻ (parental configuration) of *mec1-Δ* diploid (YB324), (B) Spontaneous His⁺ nonreciprocal translocation derived from *mec1-21* diploid (YB325), (C) Spontaneous His⁺ recombinant from *mec1-21* diploid (YB325) containing novel rearrangements, (D) Spontaneous His⁺ recombinant from *mec1-Δ* diploid (YB324) containing novel rearrangements, (E) UV-induced His⁺ recombinant from *mec1-Δ* diploid (YB324) containing novel rearrangements, (F) UV-induced His⁺ recombinant from *mec1-Δ* diploid (YB325) containing reciprocal translocation, (G) UV-induced His⁺ recombinant from *mec1-Δ* diploid (YB324) containing nonreciprocal translocation, (H) His⁻ (parental configuration) of *mec1-Δ* diploid (YB324).

Table 1

Yeast strains

Strain	Genotype	Source (Synonym)
YA102	MATa <i>ura3-52 his3-Δ200 lys2-801 trp1-Δ1 ade2-101</i>	M. Carlson (MCY727)
YA165	MATa <i>ura3-52 his3-Δ200 trp1-Δ1 leu2-Δ1</i>	F. Winston (FY250)
YA166	MATa <i>ura3-52 his3-Δ200 trp1-Δ1 leu2-Δ1</i>	F. Winston (FY251)
YA184	MATa <i>trp1-1 leu2-3,112 his3-11,15 ura3-1 can1-100 sml1::URA3 rad53::HIS3 RAD5</i>	R. Rothstein (W2105-17B)
YA185	MATa <i>trp1-1 leu2-3,112 his3-11,15 ura3-1 can1-100 mec1-Δ::TRP1 sml1::HIS3 RAD5</i>	R. Rothstein (U963-61A)
YA195	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 sml1::KanMX</i>	ResGene (512)
YA196	MATa/MATa <i>his3-Δ1/- leu2Δ0/- ura3Δ0/- met15Δ0/+ lys2Δ0/- mec1::KanMX/+</i>	ResGene (23275)
YA197	MATa <i>ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1 can1-100 mec1-21</i>	S. Elledge (Y620)
YB314	MATa <i>ura3-52 his3-Δ200 lys2-801 trp1-Δ1 gal3⁻ mec1-21</i>	Derived from cross of YB312 × YA166
YB315	MATa <i>ura3-52 his3-Δ200 ade2-a lys2-801 trp1-Δ1 gal3⁻</i>	Derived from YA102
YB316	MATa <i>ura3-52 his3-Δ200 ade2-a lys2-801 trp1-Δ1 gal3⁻ mec1-21</i>	Derived from cross of YB315 × YB314
YB317	MATa <i>ura3-52 his3-Δ200 ade2-a lys2-801 trp1-Δ1 gal3⁻ sml1::KanMX</i>	<i>sml1::KanMX</i> disruption in YB315
YB321	MATa <i>ura3-52 his3-Δ200 ade2-a lys2-801 trp1-Δ1 gal3⁻ sml1::KanMX mec1-Δ::TRP1</i>	<i>mec1-Δ::TRP1</i> disruption in YB317
<u>Strains to monitor translocations and heteroallelic events^d</u>		
YB109	MATa <i>ura3-52 his3-Δ200 ade2-101 trp1-Δ1 gal3⁻ leu2-3,112</i> <i>GAL1::his3-Δ 5' trp1::his3-Δ3'::HOcs lys2⁻ (leaky)</i>	This laboratory
YB318	MATa <i>ura3-52 his3-Δ200 ade2-n trp1-Δ1 gal3⁻ leu2-3,112</i> <i>GAL1::his3-Δ 5' trp1::his3-Δ3'::HOcs lys2⁻ (leaky)</i>	Derived from YB109
YB319	MATa- <i>inc ura3-52 his3-Δ200 ade2-n trp1-Δ1 gal3⁻ leu2-3,112</i> <i>GAL1::his3-Δ 5' trp1::his3-Δ3'::HOcs lys2⁻ (leaky) mec1-21</i>	Derived from cross of YB313 × YB318
YB320	MATa <i>ura3-52 his3-Δ200 ade2-n trp1-Δ1 gal3⁻ leu2-3,112</i> <i>GAL1::his3-Δ 5' trp1::his3-Δ3'::HOcs lys2⁻ (leaky) sml1::KanMX</i>	<i>sml1::KanMX</i> disruption in YB318
YB322	MATa <i>ura3-52 his3-Δ200 ade2-n trp1-Δ1 gal3⁻ leu2-3,112</i> <i>GAL1::his3-Δ 5' trp1::his3-Δ3'::HOcs lys2 (leaky) sml1::KanMX mec1-Δ::TRP1</i>	<i>mec1-Δ::TRP1</i> disruption in YB320
YB324	YB321 × YB322	This laboratory
YB325	YB316 × YB319	This laboratory
YB348	YB315 × YB318	This laboratory
<u>Strains to monitor SCE^d</u>		
YB163	MATa- <i>inc ura3-52 his3-Δ200 ade2-101 lys-801 trp1-Δ1 gal3-trp1::[his3-Δ3'::HOcs, his3-Δ5']</i>	This laboratory
YB204	MATa <i>leu2-Δ1</i>	This laboratory

Strain	Genotype	Source (Synonym)
YB311	<i>MATa-inc mec1-21</i>	10 th backcross of Y620 with YB163
YB312	<i>MATa mec1-21</i>	10 th back cross of Y620 with YB163
YB326	<i>MATa-inc sml1::KanMX</i>	<i>sml1::KanMX</i> disruption in YB163
YB327	<i>MATa-inc sml1::KanMX mec1-Δ::TRP1</i>	<i>mec1::TRP1</i> disruption in YB326
YB328	<i>MATa rad52::KanMX</i>	<i>rad52::KanMX</i> disruption in YB204
YB347	<i>MATa-inc mec1-21 rad52::KanMX</i>	from cross of YB329 with YB328
YB338	<i>MATa-inc sml1::URA3</i>	<i>sml1::URA3</i> disruption in YB163
YB339	<i>MATa-inc sml1::URA3 mec1-Δ::KanMX</i>	<i>mec1::KanMX</i> disruption in YB338
YB352	<i>MATa-inc sml1::URA3 mec1-Δ::TRP1</i>	<i>mec1::TRP1</i> disruption in YB338
YB368	<i>MATa-inc len2-Δ1 mec1-21</i>	from cross of YB311 × YB165
YB378	<i>MATa-inc1 mec1-21</i> + pWL25	Ura ⁺ transformant of YB311
YB381	<i>MATa mec1-21</i> + pJA98 (<i>GAL10::RAD53</i>)	Ura ⁺ transformant of YB312

^a All strains listed below YB163 have the same genotype as YB163 unless indicated. Mating type is added for clarity. YB333 and YB334 may contain either *ura3-52* or *ura3Δ10*, and *lys2Δ10* or *lys2-801*.

Table 2
Stimulation of SCE by HO-induced DSBs in *mecI* mutants

Genotype ^a (Strain)	% Viability after HO induction ^b	His ⁺ recombinants/Trp ⁺ CFU×10 ⁵		After HO induction ^d	Fold increase ^e
		Before HO induction ^c			
Wild type (YB163)	79 ± 1	8.4 ± 1.0		82 ± 5	10
<i>mecI-21</i> (YB311)	47 ± 9	11.4 ± 0.4		39 ± 6	3.4
<i>sml1::URA3</i> (YB338)	81 ± 14	7.8 ± 3.7		94 ± 30	12
<i>mecI-Δ::KanMX</i>	42.1 ± 0.02	9.1 ± 2.2		20 ± 3	2
<i>sml1::URA3</i> (YB339)					

^aFor complete genotype, see Table 1.
^bTrp⁺ CFU after HO induction/Trp⁺ CFU before HO induction × 100%
^cHis⁺ recombinants before HO induction/Trp⁺ CFU before HO induction
^dHis⁺ recombinants after HO induction/Trp⁺CFU after HO induction
^eHis⁺ frequency after HO induction/His⁺ frequency before HO induction

Table 3 Rates of spontaneous SCE in *mec1* mutants.

Strain	Genotype ^a	Rate ($\times 10^6$) ^b	Ratio ^c
YB163	<i>MEC1</i>	1.1 ± 0.1	1.0
YB326	<i>sml1::KanMX</i>	1.1 ± 0.2	1.0
YB312	<i>mec1-21</i>	6.3 ± 0.9	5.7
YB327	<i>mec1-Δ::TRP1 sml1::KanMX</i>	1.2 ± 0.2	1.1
YB378	<i>mec1-21 + RAD53</i> (pWL25)	3.3 ± 0.2	3.0

^aFor complete genotype, see Table 1.^bRate, number of events per cell division; $n \geq 3$ ^cRatio, rate of SCE in mutant/rate of SCE in wild type

Table 4
Electrophoretic karyotypes of translocations stimulated in *mecI-21* and *mecI* null mutants.

Genotype (Strain)	Agent	No. of His ⁺ recombinants containing indicated rearrangements	Reciprocal Translocations And Other	Non-reciprocal translocations	Non-reciprocal translocations and others	Other	Total
Wild type (YB110)	Spontaneous	16					16
	UV	16					16
<i>mecI-21/mecI-21</i> (YB325)	Spontaneous	5	1		1	1	8
	UV	2		5		1	8
<i>mecI-Δ/mecI-Δ</i> (YB324)	Spontaneous			3	3	2	8
	UV	3		1	3	1	8

^a *CEN2::IV* and *CEN4::II*

Table 5 Growth phenotypes of *mecI* mutants.

Genotype ^a (Strain)	Plating efficiency ^b (%)	Doubling time ^c (hours)
<i>MEC1</i> (YB163)	91 ± 5	1.9 ± 0.3
<i>mecI-21</i> (YB312)	62 ± 15	2.3 ± 0.2
<i>mecI-21rad52</i> (YB347)	22 ± 4	4.8 ± 1.1
<i>rad52</i> (YB328)	64 ± 2	2.9 ± 0.7

^aFor complete genotype, see Table 1.^bCFU/Total cell number × 100%^cAverage amount of time for logarithmically growing cells to double its cell population