Saccharomyces cerevisiae rad51 Mutants Are Defective in DNA Damage-Associated Sister Chromatid Exchanges but Exhibit Increased Rates of Homology-Directed Translocations

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ABSTRACT

Saccharomyces cerevisiae Rad51 is structurally similar to Escherichia coli RecA. We investigated the role of S. cerevisiae RAD51 in DNA damage-associated unequal sister chromatid exchanges (SCEs), translocations, and inversions. The frequency of these rearrangements was measured by monitoring mitotic recombination between two his3 fragments, his3Δ5′ and his3Δ7′::His3, when positioned on different chromosomes or in tandem and oriented in direct or inverted orientation. Recombination was measured after cells were exposed to chemical agents and radiation and after HO endonuclease digestion at his3Δ5′::His3. Wild-type and rad51 mutant strains showed no difference in the rate of spontaneous SCEs; however, the rate of spontaneous inversions was decreased threefold in the rad51 mutant. The rad51 null mutant was defective in DNA damage-associated SCE when cells were exposed to either radiation or chemical DNA-damaging agents or when HO endonuclease-induced double-strand breaks (DSBs) were directly targeted at his3-Δ5′::His3. The defect in DNA damage-associated SCEs in rad51 mutants correlated with an eightfold higher spontaneous level of directed translocations in diploid strains and with a higher level of radiation-associated translocations. We suggest that S. cerevisiae RAD51 facilitates genomic stability by reducing nonreciprocal translocations generated by RAD51-independent break-induced replication (BIR) mechanisms.

The Saccharomyces cerevisiae RAD51 gene shares sequence similarity to both higher eukaryotic genes and the Escherichia coli recA gene. The amino acid sequences of RecA and Rad51 share 30% identity in the highly conserved region that includes the Walker A and B-type nucleotide binding motifs and is responsible for oligomer formation and recombination (for review, see Shinozaka and Ogawa 1999). In vitro biochemical activities of both yeast (Sung 1994; Sung and Roberson 1995) and bacterial proteins (for review, see Kowalczykowski et al. 1994) include DNA strand exchange and the formation of protein filaments. Yeast rad51 mutants, hypersensitive to ionizing radiation, are defective in both mitotic gene conversion and meiotic recombination and yield a high percentage of inviable spores when diploid rad51 mutants undergo meiosis (for review, see Petes et al. 1991). Thus, shared biochemical properties of yeast Rad51 and E. coli RecA suggest the conservation of recombinational repair mechanisms in evolution.

Because E. coli recA plays a pivotal role in recombinational repair of DNA damage (Rupp et al. 1971), we asked whether yeast RAD51 functions in spontaneous and DNA damage-associated sister chromatid exchange (SCE). Both the DNA damage inducibility and the timing of RAD51 expression have suggested a role for eukaryotic recA homologues in SCE. Expression of yeast and higher eukaryotic homologues of RAD51 is induced at G1-S (Aboussekhra et al. 1992; Basile et al. 1992) and S and G2-M phases of the cell cycle (Chen et al. 1997), respectively. Similar to recA, the expression of yeast RAD51 is inducible by a variety of DNA-damaging agents, including X rays (Basile et al. 1992) and the alkylating agent methyl methanesulfonate (Shinozaka et al. 1992). Rad51 mutations confer X-ray sensitivity in both G1 haploids and diploids (Game 1983) and abolish radiation-induced heteroallelic recombination between homologs (Morrison and Hastings 1979). Since recombinational repair of DNA lesions by SCE is preferred over homologs (Kadyk and Hartwell 1992), the radiation sensitivity of rad51 diploid mutants may be conferred by a defect in DNA damage-associated SCE.

The participation of RAD51 in G1 recombinational repair is implied by the genetics of particular yeast UV repair mutants. Although rad51 mutants are less UV sensitive than E. coli recA mutants, yeast haploid mutants defective in both RAD1-mediated UV excision repair and RAD51-mediated recombinational repair exhibit a synergistic increase in UV sensitivity compared to the single mutants (Game and Cox 1973). Rad27 mutants, which are defective in the Fen1 (flap) endonuclease and thus accumulate unprocessed 5′ single strand overhangs during DNA replication (for review, see Lieber 1997),
require RAD51 for viability (Tischkoff et al. 1997; Symington 1998). These observations suggest that RAD51 participates in recombination between sister chromatids. Although the radiosensitive phenotypes of rad51 are well documented (Game 1983), there is no genetic assay that demonstrates that RAD51 participates in spontaneous recombination between sister chromatids in yeast. While recA mutants exhibit significant reductions in nearly all types of spontaneous recombination (for review, see Clark and Sandler 1994), rad51 mutations confer a differential effect on particular spontaneous mitotic recombination events. For example, rad51 mutations confer a profound reduction in heteroallelic recombination between homologs (Bai and Symington 1996), a minor reduction in intrachromosomal recombination between inverted repeats (Rattray and Symington 1995), and an increase in mitotic intrachromosomal recombination between direct repeats (McDonald and Rothstein 1994). Also, yeast DNA polymerase mutants exhibit a RAD51-independent hyperrecombination phenotype that is manifested by more SCEs at the rDNA locus (Zou and Rothstein 1997). Thus, although rad51 mutants exhibit deficiencies in both mitotic gene conversion and crossover events, rad51 mutants also exhibit some hyperrecombination events.

The seemingly contradictory rad51 recombination phenotypes are consistent with the hypothesis that mitotic recombination results from multiple genetic pathways (Rattray and Symington 1995). Recombination between inverted repeats can occur by a RAD51-independent, RAD59-dependent pathway (Bai and Symington 1996; Bartsch et al. 2000). Homolog recombination resulting from break-induced replication (BIR) has been suggested to account for the high frequency of repair of an HO-induced double-strand break (DSB) at the MAT locus in a rad51 diploid (Malkova et al. 1996). The BIR recombination model suggests that the homologous chromosome is used as a template for the initiation and elongation of DNA synthesis, resulting in the restoration of the broken chromosome by homologous recombination of distal markers to the break point. BIR may also participate in sister chromatid recombination and contribute to maintaining telomere length in strains defective for telomerase (Le et al. 1999). Although less efficient than RAD51-dependent gap repair pathways, these alternative RAD51-independent pathways may compete for the recombinational repair of DSBs (Haber 1999).

Here we analyzed the effects of rad51 disruptions on DNA damage-associated unequal SCE, inversions, and directed translocations in the yeast S. cerevisiae. In contrast to RAD51 strains, higher frequencies of unequal SCE were not obtained after rad51 mutants were exposed to DNA-damaging agents or when DNA DSBs were directly induced by HO endonuclease. Because higher frequencies of translocations generated by nonhomologous recombination have been previously observed in rad51 single and rfa1 rad51 double mutants (Chen et al. 1998), we also determined whether the defect in DNA damage-associated SCE correlated with an increase in homology-directed translocation events. We suggest that the rad51 hyperrecombination phenotype may result from BIR when G2 recombinational repair mechanisms are defective.

**MATERIALS AND METHODS**

Standard media for the culture of yeast, SC SC-TRP, SC-HIS, SD, YP, and YPD, and sporulation media are described by Sherman et al. (1986). YPL medium contains YP with 2% lactate (pH 5.5) and YPGal medium contains YP medium with 2% ultra-pure galactose (Sigma, St. Louis). Yeast transformations were performed according to Chen et al. (1992).

**Yeast strains**: Strains are listed in Table 1. One-step gene disruptions (Rothstein 1983) of RAD51 and RAD1 were made using the plasmid pΔRAD51 (Shinohara et al. 1992) and pDH23 (Higgins et al. 1985), respectively. Strains to monitor inversions and SCEs are isogenic to YAI02 (MCY727) or S288C except for the mating-type allele. The construction of plasmids to monitor SCEs and inversions has been previously described (Fasullo et al. 1998, 1999). Strains to monitor translocations were derived from YNN287 that had been twice backcrossed to S288C strains as previously described (Fasullo et al. 1994).

**Determining rates of spontaneous recombination and frequencies of DNA damage-associated recombinants**: The rates (events per cell division) of spontaneous, mitotic events that generate either SCE, inversions, or translocations were determined by the method of the median (Lea and Coulson 1949), as executed by Esposito et al. (1982), using 11 independent colonies for each rate calculation. At least three independent rate calculations were done for each strain, and the significance of the differences was determined by the Mann-Whitney U-test (Zar 1996).

Protocols used to measure the recombinogenicity of methyl methanesulfonate (MMS), 4-nitroquinoline oxide (4NQO), and UV and γ-rays have been described (Fasullo and Dave 1994; Fasullo et al. 1994). 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, and it is a calculation different from subtracting the average of all the spontaneous frequencies from the average of all the stimulated frequencies. The significance of the differences between rad51 mutants and RAD51 strains was determined using the two-tailed paired t-test (Zar 1996).

For measuring X-ray stimulation of SCE, cells were incubated on ice during irradiation at a dose rate of 120 rad/min. The X-ray radiation source was purchased from Faxitron (Wheeling, IL). After exposure to either UV or X rays, cells were preincubated for 30 min in YPD, washed twice with sterile H2O, and then plated on selective medium (SC-HIS). Statistical significance of the X-ray stimulation of SCE was determined by the nonparametric sign test (Zar 1996).

**Induction of HO endonuclease**: pG1HOT-GAL3 (Fasullo et al. 1998), containing the HO gene under GAL control, was introduced into both RAD51 and rad51 strains by selecting for Trp+ transformants. After growth in SC-TRP medium, cells were diluted 1:10 in YPLactate and incubated for a minimum
TABLE 1

Yeast strains

<table>
<thead>
<tr>
<th>Lab name</th>
<th>Genotype</th>
<th>Source (synonym)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YA102</td>
<td>MATa</td>
<td>M. Carlson (MCY727)</td>
</tr>
<tr>
<td>YA165</td>
<td>MATx</td>
<td>F. Winston (FY250)</td>
</tr>
<tr>
<td>YB125</td>
<td>MATa-inc</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB109</td>
<td>MATa-inc</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB110</td>
<td>YB109 × YA102</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB168</td>
<td>MATa-inc</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB169</td>
<td>MATa-inc</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB170</td>
<td>YB168 × YB169</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB163</td>
<td>MATa-inc</td>
<td>SCE assay in YB125</td>
</tr>
<tr>
<td>YB177</td>
<td>MATa-inc</td>
<td>rad51:URA3</td>
</tr>
<tr>
<td>YB198</td>
<td>MATa-inc</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB200</td>
<td>MATa-inc</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB201</td>
<td>YB163 × YA105</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB202</td>
<td>YB169 × YB198</td>
<td>This laboratory</td>
</tr>
<tr>
<td>YB165</td>
<td>MATa-inc</td>
<td>ICE&quot; assay in YB125</td>
</tr>
<tr>
<td>YB196</td>
<td>MATa-inc</td>
<td>rad51:URA3</td>
</tr>
<tr>
<td>YB197</td>
<td>MATa-inc</td>
<td>This laboratory</td>
</tr>
</tbody>
</table>

* Intrachromosomal exchange.

of 12 hr. At a density of 10^7 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 pGHO-T::GAL3 plasmid.

**Verification that His₃^+ recombinants result from unequal SCE or intrachromatid recombination:** Mitotic unequal SCE between his₃Δ5’ and his₃Δ3’ results in His₃^+ recombinants that contain HIS3 flanked by his₃Δ5’ and his₃Δ3’ (Figure 1; FASULLO and DAVIS 1987). Southern blot hybridization (SOUTHERN 1975) was used to detect a 4.4-kb EcoRI restriction fragment that contains this configuration of HIS3 and the his₃ fragments. The presence of the HOcs within this 4.4-kb EcoRI restriction was determined by Southern blot hybridization, using a 117-bp MATa fragment as a probe, and by polymerase chain reaction (PCR; ASHUEB et al. 1995), using primer 5’ GTTGGCGGAAAGCTGAAACTA 3’ that anneals to the HOcs and primer 5’ GGATCCGGGTACCGTGTTCTGC 3’ that anneals upstream of the HIS3 promoter present on his₃Δ3’:HOcs.

Mitotic intrachromatid recombination between inverted his₃ fragments, his₃Δ5’ and his₃Δ3’, results in His₃^+ recombinants that contain HIS3 and his₃Δ(5’,3’), a his₃ fragment lacking both 5’ and 3’ sequences (Figure 1). Southern blot hybridization (SOUTHERN 1975) was used to detect a 2.5-kb EcoRI restriction fragment that contains this configuration of HIS3 and the novel his₃ fragment.

**Chromosomal DNA gels:** Undigested yeast chromosomal DNA was resolved on contour-clamped homogeneous electric field (CHEF) gels containing 1% agarose (GUTH et al. 1986). The gels were run at 220 V (6 V/cm) for 26 hr at a 90-sec pulse time (FASULLO et al. 1998), and the temperature of the 0.5× TBE (Tris-borate EDTA) buffer was maintained between 10° and 15°. Chromosomal DNA was transferred to nylon after exposure to 60 mJ/m² of UV radiation for Southern blot analysis (SOUTHERN 1975). The 1.7-kb BamHI HIS3 fragment was used as a probe.

**RESULTS**

**Recombination assays:** To measure frequencies of SCEs, inversions, and translocations, His₃^+ recombinants were selected that result from mitotic recombination between two truncated his₃ fragments (FASULLO and DAVIS 1987; Figure 1). Strains to measure frequencies of translocations contain the his₃ fragments positioned...
**Figure 1.**—Recombination assays used in this study. Ovals represent centromeres and lines represent chromosomes. For simplicity, the left arms of chromosomes are not included. The position and orientation of the his3 recombinational substrates, which are present in strains used to measure (A) reciprocal translocations, (B) unequal SCE, and (C) inversions, are shown. An X designates potential sites of crossovers, and the resulting chromosomal rearrangement is presented. An arrow and feathers denote HIS3. As indicated on the bottom of the figure, the 5′ deletion lacks the feathers and the 3′ deletion lacks the arrow. The two regions of sequence identity shared by the his3 fragments are indicated by decorated boxes; broadly spaced diagonal lines indicate a region of 300 bp, and tightly spaced diagonal lines indicate a region of 167 bp. The 117-bp HO cut site (HOcs), as indicated by an arrow, is located between these sequences within the his3-Δ3′::HOcs fragment. The EcoRI sites in B and C are designated as an E and positioned centromere proximal to the his3-Δ3′ fragment and centromere distal to the his3-Δ5′ fragment.

On chromosomes II and IV (Fasullo and Davis 1987), while strains to measure SCEs and inversions contain the truncated fragments of his3 in tandem at the trp1 locus in either direct or inverted orientation, respectively (Fasullo et al. 1999). In all strains, there are no repeated sequences that flank the recombination substrates. The trp1::his3-Δ3′::HOcs fragment was used to directly target HO endonuclease-induced DSBs (Fasullo et al. 1998). Diploid strains that monitor translocations contain one set of chromosome II and IV homologs that do not contain recombinational substrates. Diploid strains that measure SCE are hemizygous for the recombination substrates.

The rate of spontaneous, unequal SCE is unchanged but DNA damage-associated SCE is reduced in rad51 null mutants: Spontaneous and DNA damage-associated SCE were compared in RAD51 (YB163) and rad51::URA3 (YB177) haploid strains. Although there was no difference (P > 0.05) in rates of spontaneous SCE in RAD51 [(2.3 ± 1.6) × 10⁻⁶] and in rad51 strains [(2.3 ± 0.9) × 10⁻⁶], stimulation of SCE after exposure to DNA-damaging agents was significantly reduced (Table 2). When RAD51 haploid cells (YB163) were plated on SC-HIS medium and defined aliquots of 4NQO were then diffused from the center of the plate, a halo of induced His⁺ recombinants appeared (Figure 2), and the inner radius of the halo is indicative of the toxicity of 4NQO. The ring of recombinants is indicative of recombination and will occur when there are more viable recombinants per total number of inoculated cells, although not always when there are simply more recombinants per viable cells. However, when the rad51 cells (YB177) were plated on SC-HIS and then exposed to either MMS (data not shown) or 4NQO (Figure 2), we observed spontaneous recombinants but no halo of induced His⁺ recombinants, even when lower concentrations (1 mM) of 4NQO were spotted on plates. To measure the differences in DNA damage-associated SCE between the rad51 mutant and RAD51 strains, we performed experiments in liquid medium. When asynchronous log phase cell cultures were exposed to MMS and 4NQO, the frequencies of His⁺ recombinants per viable cell were increased fourfold and sevenfold, respectively, above the spontaneous frequency in wild-type cells, but less than two-fold above the spontaneous frequency in the rad51 null mutant (Table 2). Thus, rad51 haploid mutants are defective in the MMS and 4NQO stimulation of SCE but are not defective in spontaneous SCE.

The genetic control of recombinational repair is different in diploids and haploids, and mitotic diploid-specific genes have been identified that are required for recombinational repair (Klein 1997). To confirm that the rad51 deficiency in DNA damage-associated SCE was not haploid specific, we constructed a rad51 homozygous diploid (YB202; Table 1) and DNA damage-associated SCE was measured. We observed no halo...
TABLE 2

Stimulation of SCE by DNA-damaging agents in \textit{RAD51} and the \textit{rad51::URA3} mutant

<table>
<thead>
<tr>
<th>Agent and conc.</th>
<th>Stimulation in \textit{RAD51} (YB163)\textsuperscript{a}</th>
<th>Stimulation in \textit{rad51::URA3} (YB177)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\text{His}^+/\text{CFU} \times 10^6 (survival %)</td>
<td>\text{His}^+/\text{CFU} \times 10^6 (survival %)</td>
</tr>
<tr>
<td>MMS 0 mM</td>
<td>35 ± 18 (100) 1 0</td>
<td>34 ± 21 (100) 1 0</td>
</tr>
<tr>
<td>MMS 2 mM</td>
<td>63 ± 4 (81) 1.8 33 ± 12</td>
<td>47 ± 25 (48) 1.3 14 ± 4 0.4</td>
</tr>
<tr>
<td>MMS 10 mM</td>
<td>140 ± 10 (49) 4 110 ± 10</td>
<td>36 ± 21 (10) 1.1 &lt;10 &lt;0.1</td>
</tr>
<tr>
<td>4NQO 0 \mu M</td>
<td>36 ± 17 (100) 1 0</td>
<td>49 ± 53 (100) 1 0 1</td>
</tr>
<tr>
<td>4NQO 10 \mu M</td>
<td>240 ± 120 (60) 6.7 200 ± 100</td>
<td>68 ± 64 (38) 1.4 20 ± 14 0.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} For complete genotype, see Table 1.
\textsuperscript{b} \text{His}^+ frequency w/agent/spontaneous \text{His}^+ frequency.
\textsuperscript{c} Net stimulation = \text{avg. (\text{His}^+ frequency w/agent - \text{His}^+ spontaneous frequency)} \times 10^6; N = 3.
\textsuperscript{d} Net stimulation for \textit{rad51}/net stimulation for \textit{RAD51}.

of 4NQO-induced recombinants on SC-HIS plates (data not shown) and less than a twofold increase in recombination frequencies (4.8 \times 10^{-5} average, N = 3) after \textit{rad51} diploid cells were exposed to 10 \mu M 4NQO compared to the spontaneous frequency (2.9 \times 10^{-5} average, N = 3). When a \textit{RAD51} homozygous diploid (YB201) containing the \text{his}3 recombination substrates was plated on SC-HIS and 4NQO was diffused from the center of the plate, we observed a halo of 4NQO-induced \text{His}^+ recombinants (data not shown). Thus, the \textit{rad51}-conferred deficiency in DNA damage-associated SCE also occurs in diploid strains.

Since \textit{rad51}-conferred sensitivity to DNA-damaging agents may explain the deficiency of DNA damage-associated SCE, we also measured DNA damage-associated stimulation in a \textit{rad1} (YB200) haploid mutant, which is more sensitive to 4NQO than the \textit{rad51} mutant (YB177). The enhanced recombinogenicity and toxicity of 4NQO in the \textit{rad1} mutant were evident after the \textit{rad1} cells (YB200) were plated on SC-HIS medium and aliquots of 4NQO were diffused from the center of the plate; the halo of stimulated \text{His}^+ recombinants is thicker and has a greater radius compared to similar plates containing \textit{RAD51} cells (Figure 2). After cells were exposed to 4NQO in liquid, there was a sixfold increase in the frequencies of SCE per viable cell in the \textit{rad1} mutant but no increase was observed for the \textit{rad51} mutant (Table 3). Exposure of the \textit{rad1} mutant to 10 \mu M 4NQO in liquid reduced viability to <1% of the unexposed cells, and stimulated recombination in the \textit{rad1} mutant could not be detected. These results indicate that DNA damage-associated SCE can be detected when...
RAD51 frequencies were observed after RAD51 log phase cultures of rad51/H11001 haploid was exposed to X rays or to 90 and 120 J/m² of UV; there was a modest twofold increase (P < 0.05) in the SCE frequency after HO induction in the rad51 cells (Table 3). Also, no detectable increase in the SCE frequency after HO induction was observed when cells were cultured in YPL and switched to glucose medium. Unequal SCE was confirmed by Southern blot analysis (Figure 4). To confirm that the His⁺ recombinants resulted from cleavage at the HOcs, we determined the proportion of the His⁺ recombinants that contain the HOcs at the recombination substrates. PCR analysis revealed that among Rad³⁺ His⁺ recombinants that appeared after HO induction, 15 of 20 no longer contained the HOcs at his3Δ3', as predicted if HO digestion occurred at his3Δ3':HOcs and initiated the recombination events. Southern blot analysis also confirmed that His⁺ recombinants lack the HOcs (data not shown). From the rad51 strain YB177, 10 of 10 His⁺ recombinants that appeared after HO induction still contained an HOcs at his3Δ3'. Thus, in the RAD51 strain (YB163) higher SCE frequencies after HO induction correlate with the absence of the HOcs.

**TABLE 3**

<table>
<thead>
<tr>
<th>Strain and genotype</th>
<th>0 µM NQO (survival %)</th>
<th>1 µM NQO (survival %)</th>
<th>Fold increase</th>
<th>Net stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>YB163 (RAD51)</td>
<td>18 ± 4</td>
<td>49 ± 21 (91)</td>
<td>2.7</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>YB177 (rad51::URA3)</td>
<td>13 ± 1</td>
<td>25 ± 7 (65)</td>
<td>1.9</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>YB200 (rad1::URA3)</td>
<td>43 ± 9</td>
<td>280 ± 170 (49)</td>
<td>6.5</td>
<td>230 ± 180</td>
</tr>
</tbody>
</table>

*For complete genotype, see Table 1.

**Rad51 mutants are defective in HO-induced SCE but not HO-induced intrachromatid deletions:** One difficulty with comparing the radiation-associated stimulation of SCE in RAD51 and rad51 mutant strains is that the level of radiation exposure necessary to generate significant stimulation of SCE in RAD51 strains results in significant lethality in rad51 mutant strains. A SCE recombination assay was therefore developed so that a site-directed DSB could stimulate unequal SCE but not confer a high level of lethality in the rad51 strains (Figure 3). DSBs were targeted directly to the recombination substrate by inserting the HO cut site (HOcs) into the his3Δ3' fragment (FASULLO et al. 1998) and expressing HO from a galactose-regulated promoter contained on the plasmid pGHOT-GAL3. The DSB could be repaired by intrachromatid recombination, by nonhomologous end-joining (NHEJ), or by SCE (Figure 4). Both RAD51 and rad51 strains contain the MATα-inc or MATα-inc allele so that there is no HO endonuclease digestion at the MAT locus. Since the phase of the cell cycle was not synchronized, we expected that there would be significant cleavage of the chromosome in G1 cells, and thus many of the recombination events would occur by intrachromatid recombination.

Relative to uninduced cells, the expression of HO endonuclease slightly reduced viability in both RAD51 and rad51 mutant cells to comparable levels (Table 4). Cleavage of chromosome IV was monitored by Southern blots (data not shown). Whereas there was an ~11-fold increase in the SCE frequency in RAD51 cells after HO induction, there was no detectable increase in the SCE frequency after HO induction in the rad51 cells (Table 4). Also, no detectable increase in the SCE frequency was observed when cells were cultured in YPL and switched to glucose medium. Unequal SCE was confirmed by Southern blot analysis (Figure 4). To confirm that the His⁺ recombinants resulted from cleavage at the HOcs, we determined the proportion of the His⁺ recombinants that contain the HOcs at the recombination substrates. PCR analysis revealed that among Rad³⁺ His⁺ recombinants that appeared after HO induction, 15 of 20 no longer contained the HOcs at his3Δ3', as predicted if HO digestion occurred at his3Δ3':HOcs and initiated the recombination events. Southern blot analysis also confirmed that His⁺ recombinants lack the HOcs (data not shown). From the rad51 strain YB177, 10 of 10 His⁺ recombinants that appeared after HO induction still contained an HOcs at his3Δ3'. Thus, in the RAD51 strain (YB163) higher SCE frequencies after HO induction correlate with the absence of the HOcs.
in the His\textsuperscript{+} recombinants, while His\textsuperscript{−} recombinants that appeared in the \textit{rad51} mutant YB177 after HO endonuclease induction were likely generated by spontaneous recombination.

Equivalent lethality of HO-induced DSBs in \textit{RAD51} and \textit{rad51} strains suggests that alternative pathways for repair of the DSB at the \textit{HOCs}, such as intrachromosomal recombinational repair (Sugawara et al. 1995) or NHEJ (religation), are preferred pathways for DSB repair in our strain construction. Although there is a low probability that His\textsuperscript{+} recombinants can result from intrachromatid recombination (Fasullo et al. 1998), intrachromatid recombination between his3 fragments could generate His\textsuperscript{−} recombinants either by gap filling or by single-strand annealing (SSA) repair mechanisms (Figure 4). Whereas both recombination mechanisms would result in the elimination of the \textit{HOCs}, SSA would generate a single his3 fragment, which could not recombine to generate \textit{HIS3}. We therefore determined whether unselected Trp\textsuperscript{+} His\textsuperscript{−} CFU arising after HO induction can generate His\textsuperscript{+} recombinants. Of a total of 163 unselected Trp\textsuperscript{+} His\textsuperscript{−} colonies that appeared after HO induction, 75\% (28/40) from \textit{RAD51} and 76\% (93/123) from \textit{rad51} strains did not generate His\textsuperscript{+} recombinants. We speculate that some of the remaining \~25\% of the unselected Trp\textsuperscript{+} His\textsuperscript{−} colonies result from cells in which the DSB was repaired by religation; a similar percentage of religation events are observed after HO cleavage at \textit{MATα} in cells lacking \textit{HML} and \textit{HMR} (Lee et al. 1999). Among the Trp\textsuperscript{+} His\textsuperscript{−} colonies that did not generate His\textsuperscript{+} recombinants, Southern blot and PCR analysis confirmed that 10/10 Trp\textsuperscript{+} His\textsuperscript{−} colonies from both \textit{RAD51} and \textit{rad51} strains contained only a single \~1-kb his3 fragment that lacked the \textit{HOCs} (Figure 5). Thus, digestion by HO endonuclease occurred in both \textit{RAD51} and \textit{rad51} strains and efficiently stimulated SSA, but stimulated SCE only in the \textit{RAD51} strain. These results are consistent with previous investigations that
showed that SSA is RAD51 independent (Ivanov et al. 1996) and account for the high level of viability obtained after HO induction in strains containing the tandem his3 fragments.

**DNA damage-associated translocations in rad51 mutants:** We had previously observed that rad9 mutants are defective in DSB-induced SCE but exhibit increased frequencies of spontaneous and DNA damage-associated translocations (Fasullo et al. 1998). If rad51 mutants are also deficient in DNA damage-associated SCE, do rad51 mutants also exhibit higher frequencies of DNA damage-associated translocation events? A rad51 disruption was made in a haploid strain (YB109) containing his3 fragments on nonhomologs to monitor translocation events (Figure 1). We found no significant difference in the rate of spontaneous translocations in the rad51 (YB168) strain (1.2 \( \times \) 10\(^{-8}\)), compared to the wild-type strain (2 \( \times \) 10\(^{-8}\)). We also observed no increase in the frequencies of radiation-associated translocations after haploid cells were exposed to 60 J/m\(^2\) and 90 J/m\(^2\) of UV (data not shown). Since both the frequencies of surviving spontaneous and DNA damage-associated translocations are greater in diploids than haploids (Fasullo et al. 1994, 1998), we measured rates of spontaneous translocations and frequencies of DNA damage-associated translocations in rad51 diploids. There was about an eightfold increase (\( P < 0.05, N = 3 \)) in the rate of spontaneous translocations in the diploid rad51 mutant ([3.3 \( \pm \) 0.9] \( \times \) 10\(^{-7}\)) compared to the diploid RAD51 strain YB110 ([4.4 \( \pm \) 2.3] \( \times \) 10\(^{-7}\)).

![Figure 5](image-url). **Figure 5.** Southern blot analysis of His\(^+\) and His\(^-\) recombinants from RAD51 (YB163) and rad51 (YB177), which arise after HO induction. EcoRI-digested DNA was resolved on agarose, transferred to nylon membrane, and probed with 32P-labeled HIS3. The 4.4-kb EcoRI fragment is indicative of HIS3 flanked by his3-\(\Delta3\)’ and his3-\(\Delta5\)’, the 2.5-kb EcoRI fragment is indicative of the parental configuration of his3-\(\Delta3\)’ and his3-\(\Delta5\)’, and the 1.0-kb EcoRI fragment is indicative of the his3-\(\Delta(3’,5’)\) fragment. Lanes: A, His\(^-\) parental RAD51 (YB163); B, His\(^+\) recombinant from RAD51 resulting from unequal SCE; C, His\(^-\) recombinant from RAD51 resulting from SSA; D, His\(^-\) parental rad51 (YB177); E, His\(^+\) recombinant from rad51 resulting from unequal SCE; F, His\(^-\) recombinant from rad51 resulting from SSA.

We then measured the frequencies of DNA damage-associated translocations in the diploid rad51 mutant YB170 (Table 5). We observed that the frequencies of UV and ionizing radiation-associated translocations were higher in the rad51 diploid mutant, in comparison to the RAD51 diploid YB110. There was an \( \approx \)10-fold or greater increase in the radiation-associated frequencies of translocations at lower levels of radiation exposure. After exposure to 14 \( \mu \)M 4NQO, there was a <2-fold increase in the net frequencies of DNA damage-associated translocations in the rad51 diploid (1.4 \( \times \) 10\(^{-5}\) average, \( N = 2 \)) compared to the RAD51 diploid (9 \( \times \) 10\(^{-6}\) average, \( N = 2 \)). Thus, the increase in the frequencies of DNA damage-associated translocations in rad51 mutants depends on the identity of the DNA-damaging agent.

**Both reciprocal and nonreciprocal translocations are**
generated in rad51 mutants: In diploid strains, viable His\(^+\) recombinants can contain either reciprocal translocations (CEN2::IV and CEN4::II) or only one translocation (CEN2::IV) that contains HIS3 (Fasullo et al. 1998). Both reciprocal and nonreciprocal translocations have been previously observed in spontaneous and DNA damage-associated His\(^+\) recombinants in rad9 mutants, which exhibit higher frequencies of DNA damage-associated translocations (Fasullo et al. 1998). We therefore determined the electrophoretic karyotypes of spontaneous and radiation-associated His\(^+\) recombinants by CHEF and Southern blot analysis using HIS3 as a probe. Among 11 independent spontaneous recombinants, 3 contained reciprocal translocations and 8 contained a nonreciprocal translocation (data not shown). Among eight radiation-associated recombinants, one contained a reciprocal translocation, six contained nonreciprocal translocations, and one contained a novel rearrangement (Figure 6). In five of the seven radiation-associated His\(^+\) recombinants that contained nonreciprocal translocations, wild-type chromosomes IV and/or II retained his3 sequences. We therefore conclude that both reciprocal and nonreciprocal translocations can occur in rad51 mutants. Considering that reciprocal translocations predominate in Rad\(^+\) diploid strains (Fasullo et al. 1998), the generation of nonreciprocal translocations contributes to the enhanced ectopic recombination observed in rad51 mutants.

**DNA damage-associated inversions in rad51 mutants:** Since the low numbers of reciprocal translocations in rad51 diploid mutants suggested that reciprocal exchanges are not stimulated in rad51 mutants, we measured spontaneous and DNA damage-associated recombination between inverted fragments of his3 (Figure 1). Mitotic recombination between inverted fragments requires that both reciprocal products are recovered for

<table>
<thead>
<tr>
<th>Strain(^a)</th>
<th>No. of experiments</th>
<th>% viability after HO induction(^c)</th>
<th>Before HO induction(^b)</th>
<th>After HO induction(^b)</th>
<th>Fold increase(^c)</th>
<th>Before HO induction (\times 10^7) CFU (avg.)</th>
<th>Trp(^+) CFU/total CFU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD51</td>
<td>7</td>
<td>78 ± 1.4</td>
<td>8.2 ± 3.4</td>
<td>87 ± 21</td>
<td>11</td>
<td>93 ± 1</td>
<td>93 ± 2</td>
</tr>
<tr>
<td>rad51</td>
<td>7</td>
<td>77 ± 1.4</td>
<td>8.2 ± 1.7</td>
<td>7 ± 2.6</td>
<td>1</td>
<td>83 ± 8</td>
<td>75 ± 6</td>
</tr>
</tbody>
</table>

\(^a\) For complete genotype, see Table 1.  
\(^b\) His\(^+\) recombinants before HO induction/Trp\(^+\) CFU before HO induction.  
\(^c\) His\(^+\) recombinants after HO induction/Trp\(^+\) CFU after HO induction.

**TABLE 4**

<table>
<thead>
<tr>
<th>Strain(^a)</th>
<th>No. of experiments</th>
<th>% viability after HO induction(^c)</th>
<th>Before HO induction(^b)</th>
<th>After HO induction(^b)</th>
<th>Fold increase(^c)</th>
<th>Before HO induction (\times 10^7) CFU (avg.)</th>
<th>Trp(^+) CFU/total CFU (%)</th>
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</tr>
</tbody>
</table>

\(^a\) For complete genotype, see Table 1.  
\(^b\) His\(^+\) recombinants before HO induction/Trp\(^+\) CFU before HO induction.  
\(^c\) His\(^+\) recombinants after HO induction/Trp\(^+\) CFU after HO induction.

**TABLE 5**

<table>
<thead>
<tr>
<th>Agent and dose</th>
<th>Stimulation in RAD51 (YB110)(^a)</th>
<th>Stimulation in rad51 (YB170)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>His(^+)/CFU (\times 10^7) (survival %)</td>
<td>Fold increase(^d)</td>
</tr>
<tr>
<td>UV</td>
<td>3.4 ± 2.4 (100)</td>
<td>1</td>
</tr>
<tr>
<td>30 J/m(^2)</td>
<td>14 ± 10 (98)</td>
<td>4.1</td>
</tr>
<tr>
<td>60 J/m(^2)</td>
<td>39 ± 9 (97)</td>
<td>11</td>
</tr>
<tr>
<td>X rays</td>
<td>5.2 ± 0.7 (100)</td>
<td>1</td>
</tr>
<tr>
<td>1 krad</td>
<td>11 ± 7 (99)</td>
<td>2.1</td>
</tr>
<tr>
<td>2 krad</td>
<td>9 ± 5 (99)</td>
<td>1.7</td>
</tr>
<tr>
<td>4 krad</td>
<td>18 ± 8 (99)</td>
<td>3.5</td>
</tr>
<tr>
<td>6 krad</td>
<td>24 ± 4 (99)</td>
<td>4.6</td>
</tr>
<tr>
<td>8 krad</td>
<td>27 ± 13 (99)</td>
<td>5.2</td>
</tr>
<tr>
<td>γ-rays</td>
<td>1.8 ± 1.5 (100)</td>
<td>1</td>
</tr>
<tr>
<td>7.8 krad</td>
<td>79 ± 8 (94)</td>
<td>42</td>
</tr>
<tr>
<td>15.6 krad</td>
<td>190 ± 10 (79)</td>
<td>106</td>
</tr>
</tbody>
</table>

\(^a\) For complete genotype, see Table 1.  
\(^b\) His\(^+\) frequency w/agent/spontaneous His\(^+\) frequency.  
\(^c\) Net stimulation = avg. (His\(^+\) frequency w/agent – His\(^+\) spontaneous frequency) \(\times 10^7\); \(N = 3\).  
\(^d\) Net stimulation for rad51/net stimulation for RAD51.
Rates of spontaneous inversions were significantly (*his3*) increased (Table 6). Although induction of HO endonuclease elevated recombination frequencies in *RAD51* and *rad51* strains, the HO-induced recombination frequencies were about sevenfold higher in the wild type compared to the *rad51* mutant. We speculate that the loss of viability after HO induction results from the inability of the cells to repair the HO-induced DSB by SSA when the *his3* fragments are in the inverted orientation. Thus, spontaneous and DNA damage-associated recombination between inverted fragments of *his3* were also reduced in the *rad51* null mutant, indicating that *RAD51* participates in reciprocal exchange between *his3* fragments.

**DISCUSSION**

Yeast *rad51* mutants exhibit pleiotropic phenotypes including radiation sensitivity and spore inviability attributed to defects in DNA gap repair. Although the participation of *RAD51* in G2 recombinational repair and mitotic gene conversion is well documented, the mitotic hyperrecombination phenotypes of *rad51* mutants are not well understood. We measured the frequencies of spontaneous and DNA damage-associated chromosomal rearrangements, which were generated by homologous recombination between *his3* fragments in both *RAD51* and *rad51* strains. We observed that yeast *rad51* mutants exhibit (1) higher frequencies of spontaneous and DNA damage-associated chromosomal translocations, (2) reduced levels of DNA damage-associated SCE, and (3) lower frequencies of spontaneous and DNA damage-associated inversions. Whereas the *rad51*-enhanced frequencies of DNA damage-associated translocations depended on diploidy and the type of DNA-damaging agent, DNA damage-associated SCE was *RAD51* dependent regardless of the DNA-damaging agent or the strain ploidy. We suggest that the *rad51* hyperrecombination phenotype results from higher levels of BIR when G2 recombination and gap repair mechanisms are defective in *rad51* mutants.

**Enhanced frequencies of nonreciprocal translocations are consistent with a BIR model for *rad51*-independent recombination:** BIR is initiated by DSBs when the replication apparatus cannot bypass DNA single-strand breaks resulting in the collapse of the replication fork (Michel 2000) or when a DSB is not repaired by more efficient gap repair mechanisms (Malkova et al. 1996). DNA synthesis is initiated when the 3′ end of the broken chromatid invades an intact template, such as a sister chromatid or homolog. However, if the 3′ end of the broken chromatid contains sequence homology to a repeated sequence and invades a repeated sequence located at an ectopic site, BIR may generate chromosomal rearrangements, such as a nonreciprocal translocation (Bosco and Haber 1998).

**BIR-mediated recombination between *his3* sequences**

...
DNA Damage-Associated Recombination in Yeast rad51 Mutants

on chromosomes II and IV would result in the formation of nonreciprocal translocations and the conservation of wild-type chromosomes II and/or IV (Figure 6) and explain the lower proportion of reciprocal translocations observed among the total homology-directed translocations in rad51 diploids. A DSB at GAL1::his3-Δ5′ could stimulate strand invasion at trp1::his3-Δ3′ and initiate replication of the long arm of chromosome IV. Since haploids containing a nonreciprocal translocation and lacking genetic information on chromosomes II or IV would be inviable, BIR may explain the occurrence of higher frequencies of spontaneous and DNA damage-associated translocations in rad51 diploids but not in rad51 haploids.

We suggest that the failure to repair DSBs by gap repair in rad51 mutants may lead to the persistence of chromosomal breaks that could initiate recombination between nonhomologs, generating more nonreciprocal translocations (Figure 7). Since rad51 mutants are defective in DNA damage-associated homolog recombination (Morrison and Hastings 1979), we cannot prove that the rad51 defect in DNA damage-associated SCE is the direct cause of the higher levels of translocations. The persistence of some types of DNA adducts after several rounds of cell division, such as those generated by 4NQO (Daza et al. 1992), implies that some DNA lesions are more easily bypassed by DNA polymerases and may poorly initiate BIR. Thus, some DNA-damaging agents may stimulate similar frequencies of translocations in RAD51 and rad51 diploids because of efficient DNA damage tolerance mechanisms for particular DNA adducts.

The decrease in the frequency of radiation-associated translocations in rad51 diploids after exposure to 15.6 krad of γ-rays (Table 5) is consistent with observations that induction of DSBs at MAT in rad51 diploids results in a significant percentage of inviable cells; this lethality may result from the formation of recombination intermediates that interfere with normal DNA replication (Malkova et al. 1996). We speculate that, similar to HO-induced DSBs, some radiation-induced DSBs would initiate BIR and generate lethal recombination intermediates in S phase; thus, a greater number of such intermediates may occur after exposure to 15.6 krad of γ-rays and decrease the frequency of nonreciprocal translocations. BIR-mediated ectopic recombination may be restricted to late S or G2 phases of the cell cycle when recombination intermediates do not interfere with chromosomal replication.

Reduced frequencies of spontaneous and DNA damage-associated inversions generated by recombination between inverted his3 fragments support the idea that rad51 mutants are defective in reciprocal exchange (Rattray and Symington 1995; Bartsch et al. 2000). RAD51-independent BIR mechanisms would be unlikely to generate chromosomal inversions because BIR, if initiated by the invasion of a 3′ end of a broken chromatin on an inverted sequence, could generate a dicentric...
chromosome. Thus, the RAD51 dependence of reciprocal exchange between inverted sequences supports the notion that higher frequencies of spontaneous and DNA damage-associated translocations in rad51 mutants do not result from a RAD51-independent reciprocal exchange pathway.

Spontaneous unequal SCE is RAD51 independent but DNA damage-induced SCE is defective in rad51 mutants: The RAD51 independence of spontaneous unequal SCE and the RAD51 dependence of DNA damage-associated unequal SCE indicate that there are two recombination pathways involved in generating unequal SCE in yeast. Minor RAD51-independent, RAD59-dependent gap repair and BIR pathways (Bartsch et al. 2000) may be responsible for RAD51-independent SCE. Since rad51 mutants did not exhibit higher levels of unequal SCE resulting from HO-induced DSBs, we would suggest that DSB-initiated gap repair mechanisms that generate spontaneous SCE events are also RAD51 dependent. Thus, we speculate that the majority of spontaneous unequal SCE occurs by BIR.

Our results indicate that diverse DNA-damaging agents, such as UV, MMS, 4NQO, and X rays, stimulate SCE by RAD51-dependent recombination mechanisms. DSBs may be indirectly formed in an attempt to repair either MMS-, 4NQO-, or UV-induced DNA lesions (Kadyk and Hartwell 1993; Friedberg et al. 1995) and may stimulate recombination by RAD51-dependent gap repair. When UV-induced pyrimidine dimers cannot be excised, as in rad1 mutants, DNA replication is necessary to create the recombinogenic lesion; this is referred to as replication-dependent recombination (Kadyk and Hartwell 1995). The enhanced 4NQO stimulation of unequal SCE observed in the rad1 mutant is also likely to occur by replication-dependent recombination. Considering that rad1 rad51 double mutants exhibit a synergistic sensitivity to UV compared to the single mutants (Game and Cox 1973), we would suggest that replication-dependent SCE is also RAD51 dependent.

We have yet to identify DNA-damaging agents that directly stimulate RAD51-independent BIR. Although X rays can generate single-strand breaks (Friedberg et al. 1995) that could stimulate BIR, observations that the highest levels of X-ray-induced SCEs occur in G2 after replication is completed (Kadyk and Hartwell 1992) suggest that BIR is not the predominant mechanism by which X rays stimulate SCE.

**Comparison of the genetic instability phenotype of rad51 and rad9 mutants:** Both rad51 and rad9 mutants exhibit no change in the spontaneous rate of SCE, a decrease in radiation-associated SCE, and an increase in radiation-associated nonreciprocal translocation events (Fasullo et al. 1998). We suggest that the rad9-enhanced frequencies

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**Figure 7.—A proposed model of how defective gap repair in rad51 mutants could enhance BIR, resulting in the generation of a nonreciprocal translocation involving chromosomes II and IV (Figure 6). Each line represents a single strand of DNA, and the arrow indicates the 3’ end. Chromosomes II and IV are designated by roman numerals. (Left) The DSB occurs at a site on the sister chromatid after polymerase progression and initiates SCE by gap repair (Szostak et al. 1983). (Right) The DSB occurs as a consequence of polymerase progression past a single-strand nick. Replication is then reinitiated after the 3’ end of the broken chromatid invades the intact sister chromatid. Non-reciprocal translocations can be generated by BIR if the 3’ end of the broken chromatid invades an intact nonhomologous chromosome. The dashed arrow indicates the possibility that a DSB, which cannot be repaired by gap repair in a rad51 mutant, can initiate BIR and generate a nonreciprocal translocation.
of translocations result from the failure to arrest the cell cycle at the G2 checkpoint; the segregation of damaged chromatids thus stimulates more translocations in the next cell cycle (Fasullo et al. 1998). In comparison to rad9 mutants, rad51 mutants still exhibit RAD9-mediated G2 arrest but are deficient in DNA damage-associated SCE when cells are exposed to either MMS or UV, which are known to stimulate replication-dependent SCE (Kadyk and Hartwell 1993; Engelward et al. 1998). Thus, we speculate that DNA damage-associated translocations result from more chromosomal breaks generated in S phase in rad51 mutants. Further experiments are in progress to determine whether rad9 rad51 double mutants would exhibit synergistic increases in the frequencies of translocations compared to the single mutants.

**Comparisons with other assays to measure ectopic recombination:** We measured frequencies of specific chromosomal rearrangements by selecting for mitotic recombination between truncated his3 fragments and thus avoided screening a large background of mitotic gene conversion events. However, other methods for measuring frequencies of homology-directed chromosomal rearrangements have relied on measuring mitotic gene conversion events associated with exchange of flanking markers. For example, Lifshitz et al. (1995) have shown that ectopic recombination between yeast Ty1 elements is RAD51 dependent. Our observation that rad51 mutants were defective in reciprocal exchange between his3 fragments is consistent with previous conclusions based on measurements of gene conversion and associated crossover events between an ade2 allele and an ade2 fragment in rad51 mutants (Rattray and Symington 1995). It will be important to determine whether the rad51-enhanced frequencies of homology-directed translocations depend on the length of the repeated sequences or would be observed when gene conversion and associated crossovers were measured between repeated sequences on nonhomologs.

**Comparison to other recA/RAD51 homologues:** We speculate that other eukaryotic recA/RAD51 homologues may function in DNA damage-associated SCE and control genetic stability. The cell-cycle regulation of these genes may facilitate sister chromatids as substrates for recombinational repair. Recently, it has been shown that RAD51-dependent homologous recombination is involved in SCE in vertebrate cells (Morrison et al. 1999; Sonada et al. 1999). In addition, mutants defective in XRCC1 and XRCC2, mammalian homologues of yeast RAD51, exhibit higher frequencies of chromosomal aberrations (Cui et al. 1999). Thus, products of mammalian tumor suppressor genes that associate with human Rad51, such as BRCA1 (Scully et al. 1997), may facilitate SCE and reduce DNA damage-associated mutation or ectopic recombination.

**Summary:** We have defined two new phenotypes for rad51 mutants: (1) defective DNA damage-associated SCE and (2) a higher spontaneous rate of homology-directed translocations. Thus, this is the first study to correlate a defect in DNA damage-associated SCE with an increase in homology-directed chromosomal translocations in rad51 mutants. It will be important to determine whether mutations in other recA/RAD51 homologues confer similar phenotypes.

We thank Debra Bressan for X-ray irradiation protocols and acknowledge John Bissonette and Rohan Samarawkoon for insightful observations when this project was initiated. We thank Dilip Nag and Robert Bauchwitz for critical comments. Doug Bishop for the rad51 disruption plasmid, and Brenda Boggs for technical assistance. This work was supported by U.S. Public Health Service grant CA-70105 from the National Institutes of Health.

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