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RAD59* is Required for Efficient Repair of Simultaneous Double-Strand Breaks Resulting in Translocations in *Saccharomyces cerevisiae

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Abstract

Exposure to ionizing radiation results in a variety of genome rearrangements that have been linked to tumor formation. Many of these rearrangements are thought to arise from the repair of double-strand breaks (DSBs) by several mechanisms, including homologous recombination (HR) between repetitive sequences dispersed throughout the genome. Doses of radiation sufficient to create DSBs in or near multiple repetitive elements simultaneously could initiate single-strand annealing (SSA), a highly-efficient, though mutagenic, mode of DSB repair. We have investigated the genetic control of the formation of translocations that occur spontaneously and those that form after the generation of DSBs adjacent to homologous sequences on two, non-homologous chromosomes in *Saccharomyces cerevisiae*. We found that mutations in a variety of DNA repair genes have distinct effects on break-stimulated translocation. Furthermore, the genetic requirements for repair using 300 bp and 60 bp recombination substrates were different, suggesting that the SSA apparatus may be altered in response to changing substrate lengths. Notably, *RAD59* was found to play a particularly significant role in recombination between the short substrates that was partially independent of that of *RAD52*. The high frequency of these events suggests that SSA may be an important mechanism of genome rearrangement following acute radiation exposure.

Keywords

Homologous recombination; double-strand breaks; single-strand annealing; translocation; repetitive sequences

1. Introduction

Acute doses of ionizing radiation, either through accidental [1,2] or therapeutic [3] exposure, have been shown to generate a diverse spectrum of genomic aberrations, with the amount of DNA damage increasing with increases in the radiation dose [4,5,6]. This damage includes double-strand breaks (DSBs) that need to be repaired for a cell to survive [7,8]. Repair can occur either through pathways that utilize HR between homologous sequences or through mechanisms that require little to no homology [9,10,11]. Following exposure to ionizing

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radiation, strains of the yeast *Saccharomyces cerevisiae* that lack proteins in the Rad52 epistasis group inefficiently repair DSBs and display significant loss of viability [7,12,13], emphasizing the importance of the homologous recombination (HR) pathway for radiation resistance. In contrast, cells lacking components of the non-homologous end-joining (NHEJ) pathway do not exhibit this sensitivity [11]. The HR and NHEJ apparatuses in yeast have homologous counterparts in higher eukaryotes, where both pathways appear important for radiation resistance [11,14,15,16,17].

Following the creation of a DSB, homologous sequence is sought out, typically from the sister chromatid or homologous chromosome, for use as a template for repair [10]. It has been shown that the HR machinery can also utilize homologous sequences located on non-homologous chromosomes [18,19]. Since evidence of such promiscuous interactions appear infrequently in normal cells it is clear that there are mechanisms to prevent them, thus maintaining the integrity of the genetic material. In cancerous and diseased cells, however, translocations, insertions, deletions, duplications, and truncations are frequently observed [20,21,22] suggesting that these mechanisms have been abrogated such that inappropriate templates are utilized for repair.

The numerous repetitive elements dispersed throughout all eukaryotic genomes are likely candidates for these inappropriate templates. In *S. cerevisiae*, there are over 100 copies of the 250 bp delta repeat [23,24]. Spontaneous recombination events between these and other repetitive elements have been shown to lead to genome rearrangements [25,26,27]. In humans, the 300 bp Alu repeats are the most abundant repetitive element with approximately 10^6 copies identified to date. These repeats are spaced an average of 4 kb apart, with each one possessing from 70 to 98% homology to a consensus sequence [28]. Evidence is accumulating that these repeats are involved in the genomic instability associated with some diseases [21,29]. Previous studies have examined HR involving substrates that are similar in size to these repetitive elements and have shown that translocations can occur spontaneously, while DSBs created at one or both substrates greatly increase their frequency [19,30,31,32].

In this study, we have monitored repair following the generation of DSBs at multiple loci in diploid *S. cerevisiae* cells, as might occur following exposure to ionizing radiation, and found a physical translocation involving homologous sequences on two chromosomes to be the major product. The frequency at which this translocation is recovered, the non-conservative nature of the recombination event, and the genetic requirements for recovery have implicated single-strand annealing (SSA) [33,34], a highly efficient form of HR. Furthermore, we have found that the genetic control of SSA varies with the lengths of the substrates, suggesting that the amount of homology available for repair may determine the nature of the apparatus that is engaged. Most notably, Rad59 appears to play a particularly important role in driving translocation formation with short substrates. The function of Rad59 in translocation formation is distinct from that of its paralog [35], the central homologous recombination protein Rad52, suggesting that Rad59 can act in multiple contexts in HR.

2. Materials and Methods

2.1. Strain and plasmid construction

Standard techniques for yeast growth, genetic manipulation and plasmid construction were used in this study [36,37]. Isogenic yeast strains were used throughout the study. The *his3-Δ3'-HOCs* allele was constructed and introduced into the *HIS3* locus of chromosome XV as follows: pUC-*HIS*, which carries the 1.8 kb wild-type *HIS3* sequence cloned into the *Bam*HI site of pUC18 [38], was used to create pLAY498 by removing the *Kpn*I site from the polylinker. Subsequently, a 127 bp *Kpn*I/*Xho*I fragment from pLAY97 [39] carrying the HOCs was used to replace a 238 bp *Kpn*I/*Xho*I fragment of pLAY498 containing the final 41 bp of the *HIS3*

coding sequence, creating *his3-Δ3'-HOcs* on pLAY500. The construct was excised from pLAY500 by digestion with *Bam*HI and inserted into the *Bam*HI site of the integrating plasmid YIp356R [40] to create pLAY504. pLAY504 was linearized with *Msc*I to target the *HIS3* locus upon transformation. Insertion of the plasmid at the *HIS3* locus in Ura⁺ transformants was confirmed by genomic blot analysis. Following selection on 5-FOA [41], and screening for histidine auxotrophs, genomic blot analysis was performed to confirm the presence of *his3-Δ3'-HOcs* at the *HIS3* locus (A. Bailis, unpublished data).

The *his3-Δ5'-(300)-HOcs* cassette that shares 311 bp of coding sequence homology with *his3-Δ3'* was created by PCR. The cassette was made in three-steps. First, a 145 bp fragment containing the 117 bp HOcs with *HIS3* homology at the 3' end was amplified using a primer complementary to the 5' end of the HOcs sequence from pLAY97 (primer-1: 5'-ATA TCC CGG GGG TAC CCA ACC ACT CTA C-3') and another complementary to the 3' end of the HOcs plus 18 bp corresponding to nucleotides 311–328 of the *HIS3* coding sequence (primer-2: 5'-CCA GTA GGG CCT CTT TAA CTC GAG GGG GAT CTA AAT-3'). Second, a 574 bp *his3-Δ5'-(300)* fragment was amplified using a primer complementary to the *HIS3* sequence in the previous fragment (primer-3: 5'-TTA AAG AGG CCC TAC TGG-3') and a 28mer containing a *Sma*I site and sequence just downstream of the *Xho*I site between the *HIS3* and *DED1* loci on chromosome XV (primer-4: 5'-GTG TCC CGG GCT CGA GTT CAA GAG AAA A-3'). Finally, the 701 bp *HOcs-his3-Δ5'-(300)* cassette was amplified using the two previous fragments as templates with primer 1 and primer 2. pLAY19 was created by inserting a 2.8 kb *Sal*I genomic clone of *LEU2* into the *Sal*I site of pGEM2 (Promega). The *HOcs-his3-Δ5'-(300)* fragment was digested with *Sma*I and cloned into the *Eco*RV site in the coding sequence of *LEU2* on pLAY19 to create pLAY530. The *leu2::HOcs-his3-Δ5'* fragment was excised using *Bam*HI/*Sna*BI and blunt-end cloned into YIp356R to create the integrating plasmid pLAY532. pLAY532 was linearized with *Afl*III to target it to the *LEU2* locus following transformation. Strains testing positive for plasmid insertion by uracil prototrophy were plated to 5-FOA media to select for plasmid loss. Leucine auxotrophs were subjected to genomic blot analysis to confirm insertion of the *HOcs-his3-Δ5'-(300)* construct into the *LEU2* locus (A. Bailis, unpublished).

A similar approach was used to create the *HOcs-his3-Δ5'-(60)* cassette bearing 60 bp of coding sequence homology with *his3-Δ3'*. A 145 bp fragment containing the HOcs and 18 bp of DNA corresponding to nucleotides 563→579 of the *HIS3* coding sequence was amplified using primer-1 described above and primer-5 (5'-GAA CGC ACT CTC ACT ACG CTC GAG GGG GAT CTA AAT-3') using the HOcs sequence on pLAY97 as template. A 326 bp fragment corresponding to sequences from nucleotide 562 of the *HIS3* coding sequence to just beyond the *Xho*I site that lies between *HIS3* and *DED1* was amplified using primer-4 described above and primer-6 (5'-CGT AGT GAG AGT GCG TTC-3') using pUC-*HIS* as a template. The final 452 bp *HOcs-his3-Δ5'-(60)* fragment was amplified by using primer-1 and primer-4 with the 145 bp and 326 bp fragments as substrates. Following digestion with *Sma*I, this fragment was cloned into *Eco*RV digested pLAY19 to create the plasmid pLAY522. The *leu2::HOcs-his3-Δ5'-(60)* fragment was excised from pLAY522 by digestion with *Bam*HI and *Hind*III and inserted into *Bam*HI and *Hind*III digested YIp356R to create pLAY524. pLAY524 was linearized by digestion with *Afl*III to target insertion to the *LEU2* locus. Verification of insertion and subsequent manipulations were as described above for *HOcs-his3-Δ5'-(300)*.

A galactose-inducible HO endonuclease expression cassette marked with *KAN-MX* was inserted into the *TRP1* locus of chromosome IV. The 3.0 kb *Pvu*II/*Hpa*I fragment containing the cassette was removed from pGHOT [42] and cloned into the *Eco*RV site of pBluescript II (Stratagene) to create pLAY433. A 1.5 kb *Bam*HI fragment containing the *KAN-MX* gene was excised from pLAY415 and cloned into the *Bgl*III site of pUC-*TRP* [38], which places it just 3' to the *TRP1* ORF to create pLAY520. Digestion of pLAY433 with *Nhe*I and *Xba*I released

a 3.0 kb *GAL-HO* fragment that was blunted with T4 DNA Polymerase (New England Biolabs) and cloned into the *EcoRV* site of pLAY520 that lies within the *TRP1* coding sequence, creating pLAY536. pLAY536 was digested with *PvuII* to release the *trp1::GAL-HO-KAN-MX* (*trp1::GHOK*) fragment which was electroporated into a *Trp*⁺ strain. G418 resistant transformants were screened for tryptophan auxotrophy and insertion of *trp1::GHOK* into the *TRP1* locus was verified by genomic blot analysis (G. Manthey and A. Bailis, unpublished data).

The *his3-Δ3'-HOcs*_(MUT) cassette, which contains an uncleavable HO-endonuclease recognition sequence, was made by plating a *MATa::LEU2* haploid strain containing both the *his3-Δ3'-HOcs* and *trp1::GHOK* cassettes to YPG medium (2% galactose, 2% bacto-peptone, 1% yeast extract). Recovered strains were sequenced to confirm mutation of the HO recognition sequence. An induction profile was performed on a mutant that had the sequence mutated from AACAGTAAAA to AACTGT-AA to confirm that the substrate was no longer cleaved (N. Pannunzio and A. Bailis, unpublished data).

The construction of the *rad1-*, *rad51-*, *rad52-*, *mre11-*, *msh2-*, *dnl4-*, and *hdf1-null* and *his3-Δ200* alleles were discussed previously [30,43,44,45,46,47,48,49]. The *rad59-null* allele was generated by transforming yeast with a PCR fragment created with primers that had 48 nucleotides complementary to the sequence immediately upstream (5'-GGG TTA CGT AGA GGA GAA GAG CAT ATT TCA GGA TAA ACA GAC AAA ATA GCG GGT GTT GGC GGG TG-3'), or downstream (5'-CAA GCA AAA TAA ATT TGC TAC TTG TGC CCT TTT TCT TTC TTT TTT TTT TGG CGC GAC GCG CCC-3') of the *RAD59* open reading frame, and 15 to 17 nucleotides complementary to sequences upstream and downstream of the *LEU2* marker in pRS415. Modification of the *RAD59* locus was verified by PCR and genomic blot analyses (G. Manthey and A. Bailis, unpublished data).

2.2. Spontaneous translocation rates

Ten mL YPD (2% dextrose, 2% bacto-peptone, 1% yeast extract) cultures were inoculated with single colonies and grown for 24 hours at 30°C. Approximately 1×10^9 cells were plated to medium lacking histidine to select for translocations. Viable counts were determined by plating dilutions to YPD medium. Rates were determined by the method of the median [50]. The 95% confidence intervals were calculated using Microsoft® *Excel*. In situations where the median rate was zero, fluctuation analysis [51] was employed. When over 90% of the trials resulted in no recombinants, a theoretical rate was determined based upon 10% recovery of histidine prototrophs with the actual rate expected to be at or below this number. Selected His⁺ recombinants were subjected to genomic blot analysis to verify the presence of wild-type *HIS3* sequence (G. Manthey and A. Bailis, unpublished data), and to chromosome blot analysis to verify the presence of translocations as described below.

2.3. HO-stimulated translocation frequencies

One mL cultures of synthetic complete (SC) medium containing 3% glycerol and 3% lactate were inoculated with single colonies and incubated for 24 hours at 30°C. Galactose was added to a final concentration of 2% to induce expression of the HO endonuclease. After four hours of induction the cells were plated to medium lacking histidine and the translocation frequency was determined by dividing the number of histidine prototrophic colonies by the total number of viable cells as determined by plating dilutions onto YPD. The median translocation frequency was determined and a 95% confidence interval was calculated using Microsoft® *Excel*. Selected His⁺ recombinants were subjected to genomic blot (G. Manthey and A. Bailis, unpublished data) and chromosome blot analyses. Southern blot analysis confirmed complete cutting of both substrates by HO endonuclease following the addition of galactose (A. Bailis, G. Manthey, unpublished).

2.4. Plating efficiencies

To determine plating efficiencies prior to and following HO induction, an aliquot of cells was removed from the overnight culture 3% glycerol, 3% lactate culture, and cell number was determined by hemocytometer count. Two hundred to 500 cells were then plated onto YPD and incubated at 30°C for two days. Galactose was added to the remaining culture to induce expression of HO endonuclease for 4 hours at 30°C. Following induction, an aliquot of cells was removed, cell number assessed by hemocytometer, 200 to 500 cell plated to YPD, and incubated at 30°C for two days. Plating efficiency is reported as the number of colonies appearing on the YPD plates divided by the number of cells plated and that quotient multiplied by 100. The median percentage $\pm$ a 95% confidence interval was calculated from at least five independent trials.

2.5. Contour-clamped homogenous electric field (CHEF) analysis

Selected His⁺ recombinants were used to prepare chromosomes in agarose plugs by an established method (<http://www.bio.com/protocolstools/protocol.jhtml?id=p1190>). Chromosomes were separated on a 1% agarose gel using a Bio-Rad CHEF-DRII apparatus with the following parameters: 1st Block: 14°C, 70 second switch time, 15 hours at 6V/cm; 2nd Block: 14°C, 120 second switch time, 11 hours at 6V/cm. Chromosomes were visualized by staining with 1 μ g/mL ethidium bromide for 30 minutes, irradiating with 60 mJoules of UV in a UV Stratalinker (Stratagene) and destaining for 30 minutes in dH₂O. Chromosomes were transferred to a positively charged nylon membrane (Hybond N+, GE Healthcare) under denaturing conditions (0.4N NaOH, 1.5M NaCl) by capillary action. Subsequently, the membranes were probed with either a 1.8 kb *Bam*HI *HIS3* genomic clone or a 2.0 kb *Sma*I/*Hpa*I *LEU2* fragment from pLAY19. The *HIS3* probe extends from 469 bp upstream of the *HIS3* coding sequence to 634 bp downstream. The *LEU2* probe extends from 406 bp upstream to 478 bp downstream of the *LEU2* coding sequence. Each DNA fragment was labeled with ³²P by random priming [52] using a Megaprime DNA labeling kit (GE Healthcare). Blots were exposed to film, and the film was developed using a Konica Minolta SRX-101A processor.

2.6. Genomic Southern Analysis

The same His⁺ recombinants used in the CHEF analysis were used to prepare genomic DNA [53]. *Bam*HI digested fragments were separated on a 1% agarose gel and transferred to a neutral membrane (Hybond N, GE Healthcare) by established methods [54]. Hybridization with *HIS3* and *LEU2* probes were done as described above.

3. Results

3.1. Development of an assay to measure interchromosomal recombination in diploids

We have designed an assay to study the events that occur following the formation of multiple DSBs within the yeast genome to better understand the genomic effects of exposure to ionizing radiation. We have used diploid cells to more closely mimic what may occur in the somatic cells of higher eukaryotes. The assay measures the frequency that an intact *HIS3* coding sequence is generated by recombination between a 3' truncated *his3* allele at the *HIS3* locus on one copy of chromosome XV (*his3-Δ3'*), and a 5' truncated *his3* allele inserted at the *LEU2* locus on one copy of chromosome III (*his3-Δ5'*) (Figure 1A). Two versions of the *his3-Δ5'* substrate were constructed. *his3-Δ5'*₍₃₀₀₎ lacks all *HIS3* information upstream of nucleotide 311 of the coding sequence, giving it 311 bp of shared homology with *his3-Δ3'*. *his3-Δ5'*₍₆₀₎ lacks all *HIS3* sequence upstream of coding nucleotide 562 of the coding sequence, giving it 60 bp of shared homology with *his3-Δ3'*. Located adjacent to each of the *his3* substrates is a 117 bp fragment of *MATa* carrying the recognition sequence for HO endonuclease. The *his3-*

$\Delta 200$ allele, deleted for the *HIS3* promoter and coding sequence [30], was present at the *HIS3* locus on the other copy of chromosome XV to prevent interaction with the *his3- $\Delta 3'$* and *his3- $\Delta 5'$* substrates.

The ability of these substrates to recombine and generate a wild-type *HIS3* gene was examined under two different conditions. Spontaneous recombination was assayed in cells lacking an HO endonuclease expression cassette as described in the Materials and Methods. Recombination following the creation of DSBs at each of the substrates was assayed in cells in which HO endonuclease is expressed from a galactose-inducible gene inserted at the *TRP1* locus on chromosome IV (Materials and Methods). HO endonuclease also generates DSBs at the *MAT* locus on the left arms of both copies of chromosome III (Figure 1B and 1C). Since all three segments of chromosome III contain sequences essential for viability, recovery of a His⁺ recombinant requires the simultaneous generation of an intact *HIS3* coding sequence and the recovery of at least one copy of all segments of chromosome III. As high doses of ionizing radiation result in chromosome fragmentation by generating multiple DSBs, this assay provides a means of studying the genome destabilizing effects of radiation exposure, and the impact of DSB repair on radiation survival.

3.2. DSBs at both substrates greatly stimulate recombination

We determined the spontaneous rate of His⁺ colony formation in order to establish a baseline for the ability of the cell to generate a functional *HIS3* gene in strains lacking the HO endonuclease expression cassette. In wild-type cells, the rate of His⁺ colony formation was 6.0×10^{-9} events/cell/generation when there was 300 bp of sequence homology between the two substrates. When this homology was shortened to 60 bp, the recombination rate decreased 35-fold to 1.7×10^{-10} events/cell/generation. These results suggest that spontaneous recombination between short repetitive sequences, such as delta elements or tRNA genes, are likely to be extremely rare events.

In contrast, cells in which HO endonuclease was transiently expressed displayed His⁺ colony formation at a frequency of 6.6×10^{-2} when the substrates shared 300 bp of sequence homology and 9.3×10^{-3} when they shared 60 bp of homology (Table 1). Interestingly, a DSB adjacent to only one of the substrates is approximately 10,000-fold less stimulatory for recombination involving both the 300 and 60 bp substrates (Table 1). These results are similar to those of previously published experiments [19,30,31], and suggest that while a DSB adjacent to a single substrate promotes more productive interaction than none at all, DSBs adjacent to both substrates greatly increases productive interaction between them.

The plating efficiency of cells prior to and following induction of HO endonuclease was assessed to determine how multiple DSBs affect survivorship. In wild-type cells where a break was initiated at both substrates, and also at the *MAT* loci on both copies of chromosome III, a modest decrease in plating efficiency from 92% to 69% was measured, but these numbers were not significantly different (Table 1). This was also true of strains in which a DSB was initiated at only one of the recombination substrates (Table 1). The potential impact of creating DSBs at the same position on both copies of chromosome III was also examined by substituting the *MATa::LEU2* allele for the wild-type *MAT* allele in the copy of chromosome III that does not contain the *his3- $\Delta 5'$* recombination substrate, allowing for one copy of chromosome III to remain unbroken. Observing no significant change in plating efficiency (Table 1) suggests that DSBs at the *MAT* locus are efficiently repaired in wild-type diploid cells. Furthermore, the recombination frequencies following DSB formation at both *his3- $\Delta 3'$* and *his3- $\Delta 5'$* in strains containing *MATa::LEU2* were not significantly different from the frequencies obtained when both *MAT* loci were wild-type (Table 1), suggesting that having breaks at both *MAT* loci does not significantly affect the recovery of His⁺ recombinants.

3.3. Spontaneous and DSB-stimulated His⁺ recombinants display evidence of distinct patterns of substrate utilization

The substantial difference in the efficiencies of the spontaneous and DSB-stimulated recombination in our assays suggested that they may have occurred by different mechanisms. We addressed this possibility by examining 15 representative His⁺ recombinants obtained spontaneously, and following DSB formation in wild-type strains bearing recombination substrates sharing 300 bp of sequence homology. Digestion of the genomic DNA with *Bam*HI, and probing with a 1.8 kb *Bam*HI *HIS3* genomic clone fragment permits substrate and product bands to be distinguished on genomic Southern blots (Figures 2A and 2B). Chromosome blot analysis was employed to determine the location of the *his3-Δ3'* and *his3-Δ5'* substrates, and the *HIS3* products among the full complement of chromosomes in the His⁺ recombinants (Figure 2C). The agarose embedded chromosomes used in this analysis were derived from the identical cell cultures used to prepare the genomic DNA for genomic Southern blot analysis (Materials and Methods). Chromosome blots were probed with the same 1.8 kb *Bam*HI *HIS3* fragment used in the analysis of the genomic Southern blots.

Genomic Southern blot analysis of the parent strain revealed the predicted 1.7 kb *his3-Δ3'* and 0.7 kb *his3-Δ200* bands from the chromosome XV homologs, and the 7.8 kb *his3-Δ5'* substrate from one copy of chromosome III (Figures 2A and 2B). The chromosome blot analysis corresponded closely (Figure 2C), revealing sequences homologous to the *HIS3* probe on chromosomes XV and III, consistent with the *his3-Δ3'* substrate and the *his3-Δ200* allele at the *HIS3* loci, and the *his3-Δ5'* substrate at the *LEU2* locus.

The genomic and chromosome blot analyses of the 15 spontaneous His⁺ recombinants (Figures 2B and 2C) revealed four distinct patterns (Sp-1 to Sp-4). Importantly, the genomic blot analysis revealed that all His⁺ recombinants possessed the 5.0 kb band indicative of an intact *HIS3* gene comprised of sequences from chromosomes XV and III (Figure 2A and 2B). Notably, in three of the four classes (Sp-1, Sp-3, and Sp-4), which represent 13/15 of the spontaneous His⁺ recombinants, both of the recombination substrates are retained subsequent to the creation of the tXV:III product, which is highly suggestive of a conservative recombination mechanism. In the remaining class (Sp-2), a 4.9 kb fragment is detected, which corresponds to the presence of the tIII:XV reciprocal product (Figure 2A and 2B). The failure to retain the parent substrates in this class suggests that they may have been used in the generation of the spontaneous reciprocal translocations. Reprobing the Sp-2 recombinants with a *LEU2* sequence confirmed that the tXV:III and tIII:XV products are comprised of sequences from both chromosomes XV and III (N. Pannunzio and A. Bailis, unpublished data), consistent with their being products of reciprocal recombination.

Chromosome blot analysis of the spontaneous recombinants (Figure 2C) revealed that the majority (12/15; classes Sp-1, Sp-2 and Sp-4) possessed the novel 0.8 Mb band corresponding to the tXV:III translocation chromosome. However, class Sp-3, while appearing identical to class Sp-4 on the genomic Southern blot did not display the tXV:III translocation chromosome on the chromosome blot. Instead, close examination of the electrophoretic karyotype of Sp-3 revealed an additional product running slightly higher than chromosome III, generating a doublet. This suggests that following the initial formation of the translocation product a subsequent rearrangement occurred that rearranged tXV:III but maintained a functional copy of *HIS3*. The 0.6 Mb tIII:XV reciprocal translocation product predicted to be in the Sp-2 class from the genomic Southern data was also confirmed. As in the genomic Southern blot, both tXV:III and tIII:XV are detected when the blot was stripped and reprobed with a *LEU2* sequence, confirming that the products are composed of sequences from both chromosomes III and XV (N. Pannunzio and A. Bailis, unpublished data).

Chromosome blot analysis also provided further evidence of the conservative nature of the spontaneous translocation events. While it was not possible to distinguish the copy of chromosome XV bearing *his3-Δ3'* from the one bearing *his3-Δ200*, we were able to detect the copy of chromosome III containing *his3-Δ5'*. Interestingly, while the *his3-Δ5'* substrate was detected in 12/15 recombinants on the genomic Southern blot (classes Sp1, Sp-2 and Sp-4; Figure 2B), the chromosome blot indicated that this substrate did not remain on an intact copy of chromosome III in class Sp-4 (Figure 2C). Instead, a chromosome that was slightly larger than the tIII:XV translocation chromosome appeared in these recombinants. Hybridization with the *LEU2* probe revealed the presence of chromosome III sequences on this novel chromosome (N. Pannunzio and A. Bailis, unpublished observations) suggesting that *his3- 5'* could be associated with it. Regardless of the location of *his3- 5'*, the apparent plasticity of the karyotypes of the recombinants further attests to the instability of the genome following translocation.

Genomic Southern blot analysis of 15 independent His⁺ recombinants recovered following HO cleavage of the *his3-Δ5'* substrate (Figure 2B) revealed three classes (SB-1 to SB-3). Like the spontaneous recombinants, all had the 5 kb *HIS3* recombinant band consistent with the tXV:III translocation. Unlike the spontaneous recombinants, however, *his3- 5'* was never retained, consistent with the cut substrate always being used in recombination. In 2/15 recombinants the *his3- 3'* substrate was retained (class SB-3), suggesting that it could be utilized without being destroyed when recombination was initiated by a DSB at the other substrate. Two of the recombinants displayed the 4 kb fragment indicative of the tIII:XV translocation (class SB-2), demonstrating that initiating recombination with a single DSB could result in reciprocal recombination events.

The chromosome blot of the single-break recombinants confirmed the presence of the canonical tXV:III translocation chromosome in all but the two SB-3 recombinants, which, instead, display a novel chromosome that is smaller than chromosome III (Figure 2C). Reprobing with *LEU2* sequence revealed the presence of chromosome III sequences on this chromosome (N. Pannunzio and A. Bailis, unpublished observations), suggesting that events subsequent to the generation of the intact *HIS3* sequence rearranged the translocation chromosome. This probe also hybridized to both the putative tXV:III and tIII:XV chromosomes observed in the SB-2 recombinants, verifying that these are reciprocal translocations.

Genomic Southern blots of 15 His⁺ recombinants recovered after the creation of DSBs at both *his3* substrates revealed that all contained the 5 kb *HIS3* sequence but that none possessed intact copies of either substrate (Figure 2B). This suggests that translocation formation following DSBs at both substrates is always non-conservative, where cutting the substrates greatly stimulates their utilization in recombination. Two of the recombinants (DB-2) also contained the 4 kb band consistent with the presence of the tIII:XV reciprocal translocation, suggesting that reciprocal translocations can arise when both substrates are broken. Both the 5 kb and 4 kb species were detected when hybridized to a *LEU2* probe, consistent with both containing sequences from chromosomes XV and III (N. Pannunzio and A. Bailis, unpublished observations). Chromosome blot analysis confirmed the presence of the tXV:III translocation chromosome in all recombinants and the tIII:XV translocation chromosome in the putative reciprocal recombinants (Figure 2C).

3.4. Spontaneous and DSB-stimulated translocation formation exhibit discrete genetic control

Having established that the rearrangements seen in the assay are translocations, we next sought to determine the genetic requirements of translocation formation. Spontaneous and DSB-stimulated translocation formation were examined in a battery of DNA repair mutants. Using the 300 bp substrates, we observed that only the central HR factor, Rad52 [7,12], and the NHEJ ligase, Dnl4 [55], were required for wild-type levels of spontaneous translocation, while

mutations in the HR repair genes *MRE11* [56] and *RAD51* [57] conferred significantly increased rates of translocation (Table 2). Few of the DNA repair mutants yielded enough spontaneous translocation events with the 60 bp substrates to draw a meaningful comparison with recombination involving the 300 bp substrates.

The frequencies of recombination following the creation of DSBs at both substrates in DNA repair mutant strains were determined in order to investigate the genetic control of translocations stimulated by multiple DSBs (Table 3). The *rad1* mutant displayed the greatest defect of all the single mutants with translocation frequencies that were 600-fold lower than wild-type with the 300 bp substrates and 93-fold lower with the 60 bp substrates. Since the structure-specific nuclease Rad1/Rad10, and the mismatch repair heterodimer Msh2/Msh3 have been shown to work together to process recombination intermediates, particularly during SSA [39,58,59,60], we also tested an *msh2* mutant and observed a substantial defect in this strain as well, with frequencies that were similar to those of the *rad1* mutant. A similar 100-fold reduction in the frequency of translocation was also observed with the 300 bp substrates in the DSB repair mutants *rad52*, *mre11*, and *rad59*. However, the *rad52* and *mre11* mutants displayed much less severe defects with the 60 bp substrates, 8-fold and 5-fold reduced frequencies respectively, while the *rad59* still displayed a 100-fold reduced frequency of translocation with the short substrates. In contrast, a mutation in *RAD51*, a DSB repair gene that has previously been shown not to be involved in SSA, [61,62], does not confer a translocation defect following DSB formation. Similarly, minimal effects on translocation frequency were observed when the genes encoding the NHEJ factors Dnl4 and Yku70 were mutated, as had been previously reported [63]. It should be noted that the effects of some of the mutations on plating efficiency following the induction of the expression of HO-endonuclease (Table 3), particularly that of *rad52*, may affect the calculation of translocation frequency. Therefore, for these mutants the effects on translocation derived from the frequencies reported in Table 3 should be considered the minimal effects.

Double mutants were examined to determine the epistasis relationships between mutations in the genes encoding the factors most important for DSB-stimulated translocation formation. Previously, combining the *rad1* and *rad52* mutations was shown to lead to a synergistic decrease in deletions by SSA, consistent with Rad1 and Rad52 occupying intersecting pathways [64]. In contrast, we observed that the translocation frequencies following DSB-formation in the *rad1 rad52* double mutant were not significantly different from those of the *rad1* single mutant with either the long or short substrates (Table 3), suggesting that Rad1 and Rad52 may participate in the same pathway toward translocation formation by SSA. Interestingly, while the *rad51* single mutant displayed no changes in translocation frequency, the *rad1 rad51* double mutant displayed translocation frequencies that were 22-fold higher with the 300 bp substrates and 88-fold higher with the 60 bp substrates than those observed with the *rad1* single mutant. This suggests that loss of Rad51 suppresses the defect conferred by loss of Rad1. Similarly, the translocation frequency with the 300 bp substrate in the *rad1 rad59* double mutant was not significantly different from that in the *rad59* single mutant and ten-fold higher than in the *rad1* mutant, indicating that *rad59* is epistatic to *rad1*. However, the translocation frequency with the 60 bp substrates in the double mutant was 19- and 33-fold higher than with the *rad59* and *rad1* single mutants, respectively, indicating that the *rad1* and *rad59* mutations were mutually suppressive with the short substrates. This complex genetic interaction may suggest that, together, Rad1 and Rad59 may obscure another, less efficient pathway toward translocation formation.

The distinct patterns of interaction of the *rad52* and *rad59* mutations with *rad1* in the DSB-stimulated translocation assays suggested that Rad59 may play a different role in translocation formation than its paralog Rad52. We further investigated this by determining translocation frequencies in *rad52 rad59* double mutants. Frequencies in the double mutant were

significantly lower than in either single mutant with both the 60 bp and 300 bp substrates (Table 3). However, the reduction was three-fold greater with the 60 bp substrates than with the 300 bp substrates, suggesting that Rad52 and Rad59 have non-overlapping roles in DSB-stimulated translocation formation, and that these functions may be more distinct for recombination between short substrates than long ones.

3.5. Characterization of recombinants in mutant strains by chromosome blot analysis

Our assay selects for the generation of an intact *HIS3* gene, but this could be carried on chromosomes other than the tXV:III translocation product, as revealed by the chromosome blot analysis of His⁺ recombinants obtained spontaneously, or after DSB formation at one substrate in wild type strains (Figure 2C). Since several of the mutants display greatly reduced frequencies of translocation following DSB formation at both substrates, the possible appearance of alternative chromosomal products was addressed by performing chromosome blot analysis on independent His⁺ recombinants obtained from single and double mutants (Table 4). Of the 127 independent His⁺ recombinants analyzed, 119 contained tXV:III, indicating that it is the major recombination product obtained following creation of DSBs at both substrates, regardless of genotype (Table 4). Indeed, there were no significant differences in the frequency that tXV:III appeared in any of the strains tested ($p > 0.05$). Chromosomal patterns other than the canonical pattern described in figure 2 appeared rarely (8/127), and were unique to individual recombinants.

4. Discussion

The risk of genome instability following exposure to high doses of ionizing radiation is quite high [1,2,3]. One particular aberration, a chromosomal translocation, appears so frequently that it can be used for dosimetry purposes [65,66] and can be stable for months after the initial exposure [67]. Due to the overwhelming evidence that chromosomal translocations are a major component of the genome instability that promotes cellular dysregulation in many types of leukemias and lymphomas [20,68], it is clear that understanding how translocations form following irradiation is of paramount importance.

Eukaryotic genomes are replete with repetitive sequences that are excellent substrates for generating translocations and other genome rearrangements. Many studies report that these sites are prone to spontaneous breakage and repair by HR [25,26,27,69]. We have developed a system that examines recombination between short tracts of homology (300 bp or 60 bp) similar in size to repetitive elements dispersed throughout the genome. The system is capable of measuring the ability of these substrates to interact spontaneously or following the creation of a DSB at one or both substrates, mimicking the likely outcomes of exposure to varying doses of ionizing radiation.

Spontaneous interaction between recombination substrates resulting in formation of a functional *HIS3* gene by chromosomal translocation seldom occurred. Examination of the spontaneous His⁺ products indicated that the translocations formed by a conservative recombination mechanism since one or both parent substrates were usually retained (Figure 2). Dependence on the central HR gene, *RAD52*, suggests that spontaneous translocations occur by a recombinational mechanism (Table 2). However, the hyper-recombinogenic phenotype of the *rad51* mutant indicates that spontaneous translocations can occur in the absence of the Rad51 strand exchange protein. Studies noting an increase in chromosome loss in *rad51* mutant strains [70,71] suggest that the increase in spontaneous translocations may result from an increase in unrepaired DSBs that stimulates inefficient recombination events.

Previous studies have found that an HO endonuclease catalyzed DSB adjacent to one of a pair of recombination substrates in haploid yeast cells is capable of generating translocations at a

substantial frequency [31]. Creation of DSBs adjacent to both substrates was shown to further stimulate translocation by HR in both yeast [19] and mammalian cells [32]. Consistent with the previous observations, we found that translocation frequencies were increased more than 1,000-fold following DSB formation adjacent to one substrate, but increased approximately 10^7 -fold with DSBs adjacent to both substrates (Table 2). Strikingly, DSBs adjacent to substrates with only 60 bp of homology were capable of generating His⁺ recombinants in nearly 1% of wild type survivors, demonstrating that translocation formation can be extremely efficient with even very short repeated sequences. Therefore, we conclude that multiple radiation-induced DSBs at or near even very short repetitive sequences would be very likely to generate translocations.

Examination of the His⁺ recombinants obtained following DSB formation at both recombination substrates revealed that they most likely occurred by a non-conservative process like SSA, as neither substrate was ever retained (Figure 2). Like DSB-stimulated deletion formation by SSA, translocation formation following DSBs at both substrates is defective in *rad52*, *rad59*, *rad1*, and *msh2* single mutants [61,62], but is not defective in *rad51* mutants [61]. Our data also indicate that *MRE11* plays an important role in DSB-stimulated translocation. Interestingly, several *mre11* mutants; *mre11-2*, *-3*, *-4*, *-11* [72], *mre11-6* [73], and *mre11-H125N* [74] displayed similar, 15- to 40-fold reductions in the frequency of DSB-stimulated translocation despite exhibiting distinct capacities to exonucleolytically process DSBs (G. Manthey and A. Bailis, unpublished data). This suggests that, like DSB-stimulated deletions [75], DSB-stimulated translocation may be independent of the role of Mre11 in promoting DSB processing, and that another function of Mre11 is required for formation of translocations by SSA. While there is precedence for NHEJ factors participating with Rad52 in Rad51-independent capture of DNA at DSBs [76], we found that mutations in either *DNL4* or *YKU70* only minimally affect translocation formation and had no effect on survivorship, suggesting that a similar pathway would have only a minor role in the formation of DSB-stimulated translocations. Overall, the physical and genetic data presented here are most consistent with these translocations occurring by SSA (Figure 3).

It has been previously demonstrated that the requirements for HR factors can vary significantly with changes in the lengths of the recombination substrates [48,60,62,77,78]. The current analysis supports this, as defects in several DNA repair genes confer considerably different effects on DSB-stimulated translocation with the 300 bp and 60 bp substrates (Table 3). The 90- to 600-fold reduction in translocation frequency conferred by the *rad1*, *mre11* and *rad52* mutations on translocation formation with the 300 bp substrates are decreased 7- to 17-fold when the 60 bp substrates are used. In contrast, the *rad59* mutation has an equivalent, 100-fold effect on translocation frequency regardless of substrate length. This may indicate that, unlike the other DNA repair factors analyzed, Rad59 may function at a step that is relatively insensitive to the amount of homology shared by the substrates.

RAD59 and *RAD52* both encode proteins with the ability to anneal homologous single strands *in vitro* [35,79], and which can act together *in vivo* [80,81]. However, differences in the biochemical characteristics of the Rad52 and Rad59 proteins [35], and the genetic characteristics of the *rad52* and *rad59* mutants [82] suggest that they are paralogs. Our genetic results support this as the *rad52 rad59* double mutant is more defective for DSB-stimulated translocation than either single mutant, particularly when the 60 bp substrates are used (Table 3). However, the best indication of how discretely Rad59 and Rad52 function in translocation is in the interactions of *rad59* and *rad52* with *rad1*. The translocation frequencies measured in *rad1 rad52* double mutants are not significantly different from those in *rad1* mutants with either the 60 bp or 300 bp substrates, demonstrating that *rad1* is epistatic to *rad52* regardless of substrate length, and suggesting that Rad1 and Rad52 act sequentially. The relationship between *rad59* and *rad1* is more complex, as *rad59* is epistatic to *rad1* with respect to

translocation with the 300 bp substrates, but *rad59* and *rad1* suppress each other in translocation with the 60 bp substrates. The latter result is particularly intriguing as it suggests that Rad1 and Rad59 work together in promoting the most efficient repair of DSBs by HR between very short sequences, but that the absence of either one leaves the other to block a less efficient SSA function.

Rad1/Rad10 is thought to function in SSA by cleaving the single-stranded 3' tails [10], such as those left over from annealing homologous sequences flanking the DSBs on chromosomes III and XV (Figure 3B). Rad59 binds single-stranded DNA [79] and has been previously implicated in the removal of 3' tails during deletion formation by SSA [62]. We suggest that Rad59 may bind to the 3' tails and facilitate their cleavage by Rad1/Rad10. In the absence of Rad1, Rad59 may remain bound to the 3' single strands and block their digestion by exonucleases. In the absence of Rad59, Rad1/Rad10 may still bind to the 3' tails but cleave inefficiently, which may also impede exonucleolytic digestion. We speculate that it is only in the absence of both that exonucleases have full opportunity to remove the 3' tails and stimulate recombination. We are pursuing a physical analysis of the role of Rad59 in translocation formation by SSA in order to address these possibilities further.

While our analysis suggests that simultaneous DSBs at repetitive genomic sequences on different chromosomes would be very likely to generate translocations by HR, such events are not frequently involved in creating the oncogenic protein fusions associated with translocations that appear in some radiation-induced cancers [83,84]. This mechanism seems more likely to contribute to the translocations that accumulate in the lymphocytes of people soon after they are exposed to radiation, which are transient [3], and so unlikely to lead directly to an oncogenic outcome. Indeed, we have found that growth of His⁺ recombinants on medium that does not select for maintenance of the translocation chromosome frequently results in its loss (N. Pannunzio, G. Manthey and A. Bailis, unpublished observations). Consequently, we suggest that these translocations, which are part of the primary response to radiation exposure [67], may contribute to tumor formation by promoting loss of heterozygosity [68,85]. Therefore, translocation formation may help initiate a cascade of genome destabilizing events that culminates in cancer.

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Figure 1. Assays for translocation formation by HR in diploids strains

(A) Both copies of chromosomes XV (black) and III (gray) are depicted (drawings not to scale). One copy of chromosome XV contains *his3-Δ3'* with an adjacent HO endonuclease recognition site, the other copy contains the *his3-Δ200* allele, shown here as a dotted line. One copy of chromosome III is unaltered at the *LEU2* locus, the other contains *his3-Δ5'* at that locus. The coding sequence of *his3-Δ5'* is directed away from the centromere to prevent the formation of a dicentric chromosome. Chromosome III also contains the *MAT* locus that has a native HO cut site. Homologous *his3* sequences (either 300 bp or 60 bp) are shown as shaded boxes. Arrows represent telomeres, circles represent centromeres. The “Spontaneous” assay system lacks the HO endonuclease expression cassette so no breaks are generated at the recombination substrates or at the *MAT* loci.

(B) The “Single-Break” assay contains a mutated HO recognition sequence at the *his3-Δ3'* substrate on chromosome XV, so that HO expression only generates a DSB at one recombination substrate, as well as at the *MAT* loci.

(C) The “Double-Break” assay has HO recognition sequences at both *his3-3'* and *his3-5'* on chromosome III, so that HO expression generates DSBs at each of the recombination substrates as well as at the *MAT* loci.

(D) In the “*MATa::LEU2*” assay the HO recognition sequence is present at each of the recombination substrates, but the *MAT* locus on one copy of chromosome III is deleted, allowing for one intact copy of chromosome III to be present throughout HO induction.

Figure 2. Analysis of DNA from *His*⁺ products through genomic Southern blotting and chromosome blot analysis reveals distinct patterns of substrate utilization depending upon the number of DSBs generated

(A) *Bam*HI fragments predicted to be detected by the 1.8 kb *HIS3* probe (black bar) before (top) and after (bottom) translocation formation by HR are shown. The fragment containing *his3-Δ3'* on one copy of chromosome XV is 1.7 kb, while the fragment containing the *his3-Δ200* allele on the other copy of chromosome XV is 0.7 kb. The fragment containing *his3-Δ5'* on one copy of chromosome III is 7.8 kb. Following DSB-induction, the *HIS3* product will be formed by a translocation (tXV:III) that joins the left arm of chromosome XV and the right arm of chromosome III, which will be revealed by a 5.0 kb fragment. Joining of the remaining arms of these chromosomes by translocation (tIII:XV) will be revealed by either a 4.0 kb (DSB-induced) or a 4.9 kb (spontaneous) fragment will be generated. Sizes of the parent chromosomes and the translocation products are shown to the right in mega base-pairs (Mb).

(B) A *HIS3*-probed Southern blot of *Bam*HI digested genomic DNA from representative *His*⁺ products obtained from the Spontaneous (Sp, lanes 2–5), Single-Break (SB, lanes 6–8), or Double-Break (DB, lanes 9 and 10) assays in wild-type cells. Lane one is DNA from a non-recombinant parent strain. Marker sizes are shown on the left side of the blot in kilobase pairs. Substrates or products contained on each fragment are indicated on the right.

(C) Agarose plugs containing whole yeast chromosomes were prepared concurrently with genomic DNA in (B) (Materials and Methods). Chromosomes were separated by CHEF. The ethidium-stained gel is shown on the left. The *HIS3* probed blot is shown on the right. Relevant chromosomes are indicated. Class designation is based upon the pattern revealed from the *HIS3*-probed blot of 15 independent His⁺ colonies followed by comparison with the pattern observed for the same recombinant on the genomic Southern blot.

Figure 3. DSBs generated near sequences that share homology can be repaired by SSA

(A) Following the creation of DSBs on chromosomes XV and III (see figure 1 for a complete description of the diagram), the *MAT* loci have been omitted for simplicity), enzymes process the breaks to generate 3' ssDNA overhangs. The sequence homology shared between the two *his3* substrates (either 300 bp or 60 bp) is shown as a shaded region.

(B) Processing of broken ends reveals the homology, allowing for the formation of a stable annealing intermediate consisting of one arm of chromosome XV and one arm of chromosome III.

(C) After removal of non-homologous sequences and ligation, a stable tXV:III translocation product is formed that is selectable by the requirement for histidine prototrophy. While our

data supports that the remaining arms of chromosome XV and III can form a tIII:XV reciprocal translocation product (not shown) it is typically lost since the diploid nature of the cell does not necessitate its retention.

Table 1Frequency of His⁺ survivors and plating efficiencies following variation in the dosage of DSBs

Assay ^c	Substrate lengths ^a		Plating Efficiency (%) ^b	
	300 bp	60 bp	Pre-Induction	Post-Induction
Spontaneous ^d	$6.0 \pm 1.6 \times 10^{-9}$	1.7×10^{-10}	N/A ^e	N/A ^e
Double-break	$6.6 \pm 1.0 \times 10^{-2}$	$9.3 \pm 1.5 \times 10^{-3}$	92 ± 10	69 ± 13
Single-break	$1.0 \pm 0.1 \times 10^{-3}$	$1.1 \pm 0.1 \times 10^{-6}$	91 ± 15	77 ± 9
<i>MATa::LEU2</i>	$5.7 \pm 0.8 \times 10^{-2}$	$8.1 \pm 1.3 \times 10^{-3}$	69 ± 13	69 ± 19

^a Recombination data is presented as the median frequency of at least 10 trials $\pm$ the 95% confidence interval (Material and Methods).

^b Plating efficiency is presented as the median percentage of at least 5 trials $\pm$ the 95% confidence interval in strains bearing 300 bp of sequence homology (Materials and Methods).

^c See Figure 1.

^d Spontaneous recombination data are presented as rates. Rates for the 300 bp assay were calculated by the method of the median while rates for the 60 bp assay were determined by fluctuation analysis (Materials and Methods).

^e Not applicable as there was no induction step.

Table 2Spontaneous translocation rates in strains sharing 300 bp of *HIS3* homology between ChrXV and ChrIII^a

Wild-Type	$6.0 \pm 1.6 \times 10^{-9}$ (1.0)
<i>rad1/rad1</i>	$7.6 \pm 2.1 \times 10^{-9}$ (1.3)
<i>msh2/msh2</i>	$5.3 \pm 0.8 \times 10^{-9}$ (0.88)
<i>rad52/rad52</i>	$<8.4 \times 10^{-10}$
<i>mre11/mre11</i>	$6.7 \pm 1.0 \times 10^{-8}$ (11.2)
<i>rad59/rad59</i>	$1.2 \pm 0.1 \times 10^{-8}$ (2.0)
<i>rad51/rad51</i>	$1.1 \pm 0.2 \times 10^{-7}$ (18.3)
<i>dnl4/dnl4</i>	7.9×10^{-10} (0.13)
<i>yku70/yku70</i>	7.4×10^{-9} (1.2)
<i>rad1/rad1 rad52/rad52</i>	$<5.0 \times 10^{-10}$
<i>rad1/rad1 rad51/rad51</i>	2.9×10^{-9} (0.48)

^a Rates reported with a 95% confidence interval were determined by method of the median [50], all others were determined by Luria-Delbrück [51]. Those rates preceded by a “≤” sign are a theoretical value due to fewer than 10% of the trials yielding recombinants (Materials and Methods). Each number represents at least 10 individual trials. Fold difference from wild-type is indicated in parentheses.

Table 3Frequency of His⁺ survivors and plating efficiencies following creation of multiple double-strand breaks by HO endonuclease^a

Genotype	Substrate lengths ^a		Plating Efficiency (%) ^b	
	300 bp	60 bp	Pre-Induction	Post-Induction
Wild-Type	$6.6 \pm 1.0 \times 10^{-2}$ (1.0)	$9.3 \pm 1.5 \times 10^{-3}$ (1.0)	92 ± 10	69 ± 13
<i>rad1/rad1</i>	$1.1 \pm 0.5 \times 10^{-4}$ (0.0017)	$1.0 \pm 0.3 \times 10^{-4}$ (0.011)	87 ± 26	58 ± 11
<i>msh2/msh2</i>	$5.3 \pm 2.1 \times 10^{-4}$ (0.008)	$2.3 \pm 0.7 \times 10^{-4}$ (0.025)	48 ± 5	32 ± 7
<i>rad52/rad52</i>	$5.5 \pm 1.0 \times 10^{-4}$ (0.0083)	$9.0 \pm 2.8 \times 10^{-4}$ (0.097)	51 ± 5	1 ± 0.6
<i>mre11/mre11</i>	$7.6 \pm 1.6 \times 10^{-4}$ (0.011)	$1.8 \pm 0.2 \times 10^{-3}$ (0.19)	60 ± 5	9 ± 5
<i>rad59/rad59</i>	$7.5 \pm 0.7 \times 10^{-4}$ (0.01)	$1.7 \pm 0.7 \times 10^{-4}$ (0.018)	72 ± 7	35 ± 6
<i>rad51/rad51</i>	$7.2 \pm 1.2 \times 10^{-2}$ (1.1)	$1.3 \pm 1.0 \times 10^{-2}$ (1.4)	42 ± 15	16 ± 13
<i>dnl4/dnl4</i>	$4.5 \pm 0.3 \times 10^{-2}$ (0.68)	$1.7 \pm 0.4 \times 10^{-3}$ (0.18)	83 ± 5	61 ± 10
<i>yku70/yku70</i>	$1.4 \pm 0.3 \times 10^{-2}$ (0.21)	$1.6 \pm 0.4 \times 10^{-3}$ (0.17)	ND ^c	ND ^c
<i>rad1/rad1 rad52/rad52</i>	$4.7 \pm 2.8 \times 10^{-5}$ (0.00071)	$3.9 \pm 3.3 \times 10^{-5}$ (0.0042)	38 ± 8	1.4 ± 1
<i>rad1/rad1 rad51/rad51</i>	$2.4 \pm 1.2 \times 10^{-3}$ (0.036)	$8.8 \pm 3.2 \times 10^{-3}$ (0.95)	102 ± 7	85 ± 11
<i>rad1/rad1 rad59/rad59</i>	$1.1 \pm 0.9 \times 10^{-3}$ (0.017)	$3.3 \pm 1.3 \times 10^{-3}$ (0.35)	ND ^c	ND ^c
<i>rad52/rad52 rad59/rad59</i>	$1.4 \pm 0.6 \times 10^{-4}$ (0.0021)	$1.4 \pm 0.4 \times 10^{-5}$ (0.0015)	56 ± 4	0.4 ± .04

^a Recombination data are presented as the median frequency of at least 10 trials ± the 95% confidence interval with the fold difference from wild-type indicated in parentheses (Materials and Methods).

^b Plating efficiencies are presented as the median percentage of at least 5 trials ± the 95% confidence interval in strains bearing 300 bp of sequence homology (Materials and Methods).

^c Not determined.

Table 4Chromosome blot analysis of His⁺ recombinants recovered from wild-type and mutant strains

Genotype	<i>HIS3</i> Homology (bp)	Number of His ⁺ products with tXV:III	Total His ⁺ products tested
Wild-Type	300	9	9
Wild-Type	60	9	9
<i>rad1/rad1</i>	300	9	10
<i>msh2/msh2</i>	300	7	9
<i>rad52/rad52</i>	300	10	10
<i>rad52/rad52</i>	60	10	10
<i>rad59/rad59</i>	300	10	10
<i>rad59/rad59</i>	60	10	10
<i>rad51/rad51</i>	300	10	10
<i>dnl4/dnl4</i>	300	9	10
<i>rad1/rad1 rad51/rad51</i>	300	6	10
<i>rad1/rad1 rad52/rad52</i>	300	10	10
<i>rad52/rad52 rad59/rad59</i>	300	10	10