Effects of Mutations of *RAD50, RAD51, RAD52,* and Related Genes on Illegitimate Recombination in *Saccharomyces cerevisiae*

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ABSTRACT

To examine the mechanism of illegitimate recombination in *Saccharomyces cerevisiae,* we have developed a plasmid system for quantitative analysis of deletion formation. A *can1 cyh2* cell carrying two negative selection markers, the *CAN1* and *CYH2* genes, on a YCp plasmid is sensitive to canavanine and cycloheximide, but the cell becomes resistant to both drugs when the plasmid has a deletion over the *CAN1* and *CYH2* genes. Structural analysis of the recombinant plasmids obtained from the resistant cells showed that the plasmids had deletions at various sites of the *CAN1-CYH2* region and there were only short regions of homology (1–5 bp) at the recombination junctions. The results indicated that the deletion detected in this system were formed by illegitimate recombination. Study on the effect of several *rad* mutations showed that the recombination rate was reduced by 30-, 10-, 10-, and 10-fold in the *rad52, rad50, mre11,* and *xrs2* mutants, respectively, while in the *rad51, 54, 55,* and *57* mutants, the rate was comparable to that in the wild-type strain. The *rad52* mutation did not affect length of homology at junction sites of illegitimate recombination.

ILLEGITIMATE recombination is the recombination that takes place between nonhomologous DNA sequences or very short regions of homology. Illegitimate recombination causes chromosome rearrangements (*e.g.,* deletion, insertion, translocation, and inversion), which can lead to cancer and hereditary disease (reviewed in MEUTH 1989).

Analysis of end product showed that short regions of homology exist at recombination sites in many cases (reviewed in ALLGOOD and SILHAVY 1988; EHRLICH 1989). For example, the presence of short regions of homology was detected at the junctions of illegitimate recombination in the *lacI-lacZ* region on an F′ plasmid in *Escherichia coli* (ALBERTINI *et al.* 1982). Short homology was also detected at the junctions of λ*bio* specialized transducing phage induced by UV light (KUMAGAI and IKEDA 1991; YAMAGUCHI *et al.* 1995). On the other hand, no homology was observed at the junctions of the majority class of the λ*bio* specialized transducing phage induced by oxolinic acid, an inhibitor of DNA gyrase (SHIMIZU *et al.* 1995).

In *Escherichia coli,* the involvement of recombination proteins in illegitimate recombination has been studied. Illegitimate recombination during formation of specialized transducing phage and deletion formation at the *tonB-trp* region on the chromosome take place independently of RecA protein (FRANKLIN 1967; INSELBURG 1967; IKEDA *et al.* 1995). A *recA* mutation affects deletion formation at the *lacI-lacZ* region on an F′ plasmid, although the role of RecA protein in illegitimate recombination is still not clear (ALBERTINI *et al.* 1982). DNA gyrase has been shown to be involved in illegitimate recombination during formation of λ*bio* specialized transducing phage (SHIMIZU *et al.* 1995). The frequency of deletion mutation by illegitimate recombination in the *ompR′lacZ* region on plasmid are increased by 30- to 100-fold in a *xonA* mutant (ALLGOOD and SILHAVY 1991).

In *Saccharomyces cerevisiae,* most of genes belonging to *RAD52* epistasis group (*RAD50-57, MRE11,* and *XRS2*) are involved in double strand break repair and homologous recombination (reviewed in PETES *et al.* 1991). Their mutants exhibit sensitivity to X-ray and defects of homologous recombination and sporulation. Recently homologues of *E. coli* RecA protein have been identified in *S. cerevisiae.* The amino acid sequences of Rad51, Rad55, Rad57, and Dmc1 proteins are homologous to that of RecA protein (ABOUSSEKHRA *et al.* 1992; BASILE *et al.* 1992; BISHOP *et al.* 1992; KANS and MORTIMER 1991; LOVETT 1994; SHINOHARA *et al.* 1992). Biochemical study shows that the Rad51 protein has DNA strand transfer activity, single-strand DNA-dependent ATPase activity and the nucleoprotein-filament-forming activity as does RecA protein (OGAWA *et al.* 1993a,b; SUNG 1994). The Rad52 product, which has a strand transfer activity (ADZUMA *et al.* 1984; OGAWA *et al.* 1993a) is also capable of associating with the Rad51 protein and is believed to work on DNA pairing reaction together with Rad51 protein (SHINOHARA *et al.* 1992; MILNE and WEAVER 1993). It seems that the roles of Rad55 and Rad57 on homologous recombination are different from that of Rad51. Analysis of *rad55* and *rad57* mutants shows that they are not as X-ray sensitive as is a *rad51*

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mutant, and UV-induced mitotic recombination is deficient in the *rad51* mutant but not in the *rad55* and *rad57* mutants (SAEKI *et al.* 1980; LOVETT and MORTIMER 1987). Dmc1 is expressed only in meiosis and does not have any functions in mitosis (BISHOP *et al.* 1992). Rad50, Mre11 and Xrs2 are required for early step in meiotic recombination, but not required for mitotic recombination (ALANI *et al.* 1990; MALONE *et al.* 1990; AJIMURA *et al.* 1992; IVANOV *et al.* 1992; JOHZUKA and OGAWA 1995). Their mutants exhibit hyper-rec phenotype in spontaneous mitotic recombination and proficiency in mating type switching (MALONE 1983; IVANOV *et al.* 1994). Rad1, belonging to Rad3 epistasis group that is involved in nucleotide excision repair, has a role in homologous recombination between direct repeats and Rad1 participates via another pathway different from Rad52-dependent pathway (KLEIN 1988; SCHIESTL and PRAKASH 1988; THOMAS and ROTHSTEIN 1989).

To understand the mechanism of illegitimate recombination in yeast, it is important to analyze the roles of *RAD* genes, which are involved in double-strand break repair and homologous recombination. These analyses may help to clarify not only the mechanism of illegitimate recombination but also the function of these proteins in homologous recombination and repair. In yeast, several systems for analysis of illegitimate recombination have been developed so far. KRAMER *et al.* (1994) analyzed end products of illegitimate recombination induced by double-strand break in *rad52* background and found that short regions of homology (1–7 bp) are involved in rejoining events. SCHIESTL *et al.* developed a system for study on illegitimate recombination and examined the effects of *rad50, 51, 52,* and *57* mutations on the integration of DNA fragments by illegitimate recombination (SCHIESTL and PETES 1991; SCHIESTL *et al.* 1993, 1994). It was shown that the integration in *rad50, 51, 52,* and *57* mutants were reduced by ~100-, 20-, 10-, and 10-fold, respectively. However, because the mechanism of the integration by illegitimate recombination may be different from that of deletion mutation or other illegitimate recombinations, it is also important to study the involvement of the *RAD* genes in the latter recombinations.

In this work, we have constructed a system for the quantitative analysis of illegitimate recombination occurring on a plasmid in *S. cerevisiae* and examined the effects of mutations of *RAD* genes, which are involved in double-strand break repair and homologous recombination. The result indicates that the Rad52, Rad50, Mre11, and Xrs2 proteins are involved in illegitimate recombination, while the Rad51, Rad54, Rad55, and Rad57 proteins are not required for the recombination.

MATERIALS AND METHODS

Strains and media: *S. cerevisiae* strain DH6.61D (*MAT***a** *can1 cyh2 trp1 his3 leu2 ura3*) was kindly obtained from J. W. SZOSTAK (Massachusetts General Hospital). All the *rad* mutants used in this study were insertion or deletion mutants constructed by one-step gene replacement method (ROTHSTEIN 1983). The *rad* mutants and plasmids for gene disruption were listed in Table 1. The gene disruption of *RAD50, RAD51, RAD54,* and *MRE11* genes was done with a *hisG-URA3-hisG* cassette (ALANI *et al.* 1987). To eliminate the *URA3* gene from the Ura$^+$ transformants, an Ura$^-$ segregant that occurred by mitotic recombination between the *hisG* direct repeats was isolated as a colony resistant to 5-fluoroorotic acid. The plasmid prad57::TRP1 had a *Sty*I-*Hin*cII fragment containing the *rad57* gene inactivated by insertion of the *TRP1* marker at a *Bgl*II site. pSL1::TRP1 contains *Sal*I fragment with *rad52* gene inactivated by insertion of a *TRP1* marker at a *Bgl*II site (N. ADACHI, Yokohama City University). The plasmid 54-HUH-B contains the *rad54* gene inactivated by replacing a *Bam*HI fragment of the *RAD54* gene with a *Bam*HI-*Bgl*II *hisG-URA3-hisG* fragment (A. SHINOHARA, Osaka University). The disruption of the *rad* genes was confirmed by Southern blotting analysis or PCR. Additionally, the disruptants were confirmed to be sensitive to γ-ray irradiation (30 krad, in a cesium source emitting 100 rad/min) and MMS (methyl methanesulfonate, 0.01%).

Liquid media used were as follows: YPD and synthetic medium (SD) containing 0.67% yeast nitrogen base and 2% glucose, supplemented with the necessary amino acids (ROSE *et al.* 1990). For solid media, 1.5% agar was added to the above media. Can (canavanine)- and Cyh (cycloheximide)-resistant cells were selected by plating yeast cells on SD plates containing canavanine sulfate (60 μg/ml) and cycloheximide (10 μg/ml), respectively.

E. coli strains used were DH1, JM109, and DH10B (SAMBROOK *et al.* 1989; GRANT *et al.* 1990).

Plasmid constructions: The techniques for construction and preparation of plasmids were as described (SAMBROOK *et al.* 1989). YCpL2 was constructed as follows. A 4.5-kb *Xho*I fragment containing the *CAN1* gene of pYECAN1-2-1 (EKWALL and RUUSALA 1991) was inserted into a *Xho*I site of pBluescript II KS+ (Stratagene) to obtain pBlueCAN1. A 1.2-kb *Sca*I-*Pst*I fragment containing the *CYH2* gene of pSZ430 (BROWN and SZOSTAK 1983) was ligated with a 5.6-kb *Sna*BI-*Pst*I fragment of pBlueCAN1 to construct pBlueCAN1CYH2. A 2.1-kb *Sac*I-*Pst*I fragment containing the *CAN1* gene of pUC-CAN1, which was constructed by ligating a 2.1-kb *Sna*BI-*Xho*I fragment of pBlueCAN1 with a *Sma*I-*Sal*I fragment of pUC118 (VIEIRA and MESSING 1987), was ligated with a 6.5-kb *Sac*I-*Pst*I fragment of pBlueCAN1CYH2 to obtain pBlueCAN1CYH2-CAN1. A 2.2-kb *Bam*HI fragment containing the *LEU2* gene of pMT34-317 (Y. SIBAGAKI, Kitazato University) and a Klenow-fragment-treated 1.3-kb *Hin*dIII fragment containing the *URA3* gene of pSZ430 were inserted into the *Bam*HI site and *Pvu*II site, respectively, of YCpN1 (NAKAYAMA *et al.* 1985), which is a pBR322 derivative containing the *ARS1, CEN3, TRP1* genes to construct YCpN1LEUURA. A 6.0-kb *Pvu*II fragment containing the *CAN1-CYH2-CAN1* genes of pBlueCAN1-CYH2CAN1 was inserted into a *Nru*I site of YCpN1LEUURA to obtain YCpD2. A 1.2-kb *Sca*I-*Pst*I fragment containing the *CYH2* gene of pSZ430 was ligated with a *Pst*I-*Eco*RV fragment of pBluescript II KS+ to construct pBlueY. A 2.6-kb *Xho*I fragment containing a *CAN1* gene and N-terminal region of the *CYH2* gene of YCpD2 was replaced with a 0.6-kb *Xho*I fragment containing N-terminal region of the *CYH2* gene from pBlueY, resulting in a plasmid, YCpL2, that contains a *CAN1* and a *CYH2* gene.

Recombination assay: YCpL2 was introduced into yeast strains by the lithium acetate method and the transformants were selected as Ura$^+$ colonies on SD (+ Trp His Leu) plates (ITO *et al.* 1983). Yeast strains transformed with YCpL2 were isolated as single colonies on SD (+ Trp His Leu) plates. Single colonies were inoculated into liquid SD (+ Trp His

TABLE 1

***Saccharomyces cerebisiae* strains**

Strain	Genotype	Plasmid for gene disruption	Origin
DH6.61D	*MAT***a** *trp1 his3 leu2 ura3 can1 cyh2*		J. W. SZOSTAK
YT211	*rad50Δ::hisG*	pNKY83 (ALANI *et al.* 1989)	This study
YT141	*rad51Δ::hisG*	pΔRAD51 (SHINOHARA *et al.* 1992)	This study
YT101	*rad52::TRP1*	pSL1::TRP1[a]	This study
YT261	*rad54Δ::hisG*	54-HUH-B[b]	This study
YT131	*rad55::LEU2*	pSTL11 (LOVETT and MORTIMER 1987)	This study
YT281	*rad57::TRP1*	prad57::TRP1[c]	This study
YT251	*mre11::hisG*	pKJ1112-S (JOHZUKA *et al.* 1995)	This study
YT171	*xrs2Δ::LEU2*	pEI40 (IVANOV *et al.* 1994)	This study
YT181	*rad1Δ::LEU2*	pRR46 (REYNOLDS *et al.* 1987)	This study

All strains are isogenic to DH6.61D. The genotype is identical to DH6.61D except where noted.
[a] N. ADACHI, Yokohama City University.
[b] A. SHINOHARA, Osaka University.
[c] This study.

Leu) medium and grown at 30° with aeration. Cells were plated on SD (+ Can Cyh Trp His Leu) plates for recombination assay and plated on SD (+ Trp His Leu) plates for viable count. The recombination rate was determined by fluctuation analysis and the data were analyzed by the methods of the median (LURIA and DELBRUCK 1943; LEA and COULSON 1948). Seven independent colonies were used for each experiment, and experiments were repeated at least five times.

Structural analysis of recombinants: Recombinant plasmid DNA, prepared from Can^R Cyh^R cells, was rescued by transfer to *E. coli recA* strain DH10B by electroporation. Two plasmids derived from each yeast clone were analyzed to ensure that a rearrangement of plasmid DNA had occurred in the yeast strain and not in the *E. coli* strain. Then the plasmids were analyzed by restriction mapping. A series of FITC-labeled primer DNAs that correspond to a region from 5′ end of the *URA3* gene to 3′ end of the *CYH2* gene on YCpL2 and a primer DNA corresponding to 5′ end of the *CAN1* gene on YCpL2 were synthesized using Gene Assembler Plus (Pharmacia). The length of all primers used in this analysis is 20 mer. The sequence and corresponding position of primers are described below in a following order. Name of primer (sequence: position on YCpL2, whose length is 13,807 bp), PRT1 (5′-TTGACCCTATCGCCACTATC-3′: 2542–2561), PRT2 (5′-TCACAACCCTCTTTCACGAC-3′: 4157–4176), PRT3 (5′-GGTAGAATTCCAAATGTTCC-3′: 6386–6367), PRT4 (5′-TTGGTATGAGATACTTCCAC-3′: 6565–6546), PRT5 (5′-CAAATCCTTGTAGAGAGCGC-3′: 6814–6795), PRT6 (5′-TAAATGTACTCTTCCATCGC-3′: 6984–6965), PRT7 (5′-CGTCTCAGGTATGTAGTTCC-3′: 7200–7181), PRT8 (5′-TTCCCAGATAGGTTCAAACC-3′: 7373–7354), PRT9 (5′-ATAAGCTTGATACTGTAACC-3′: 7548–7529), PRT10 (5′-AGCTCACTCATTAGGCACCC-3′: 7738–7718), PRT11 (5′-TAAGAGCCGCGAGCGATCCT-3′: 7940–7921), PRT12 (5′-GAATTGATTGGCTCCAATTC-3′: 8163–8144), PRT13 (5′-CTAACCAGTAAGGCAACCCC-3′: 8343–8324), PRT14 (5′-TACAGATGTAGGTGTTCCAC-3′: 8544–8525), PRT15 (5′-GTTTGGTCACTGATGCCTCC-3′: 8749–8730), PRT16 (5′-CCTTCTGTTCGGAGATTACC-3′: 8986–8967), and PRT17 (5′-GATGAGTAGCAGCACGTTCC-3′: 9157–9138). PRT1 corresponds to the *LEU2* gene region (located at 1647–2741). PRT2 corresponds to the *CAN1* region (located at 4099–5871). PRT3–PRT7 correspond to the *CYH2* gene region (located at 7241–6285). PRT8–PRT16 correspond to the region between the *CYH2* and *URA3* gene. PRT16 corresponds to the *URA3* gene region (located at 9118–9921). The location of recombination sites on plasmids was determined by PCR using a combination of PRT1 and PRT3 to PRT17 or PRT2 and PRT3 to PRT17. DNA sequences of recombination-junction sites were determined by the dideoxy chain termination methods using these primers and an Auto Read sequencing kit (Pharmacia), and the analysis was carried out with an A.L.F. DNA sequencer (Pharmacia).

RESULTS

Plasmid deletion by illegitimate recombination: Plasmid rearrangements were detected in our plasmid, YCpL2, that carries two negative selection markers, the *CAN1* and *CYH2* genes, and three positive selection markers, the *URA3, TRP1,* and *LEU2* genes on a YCp plasmid (Figure 1). Because wild-type *CAN1* and *CYH2* genes are dominant to *can1* and *cyh2* mutations, respectively, the *can1 cyh2* mutant cells, that are resistant to canavanine (Can^R) and cycloheximide (Cyh^R), become sensitive to both drugs when the cells carry the plasmid with both the *CAN1* and *CYH2* genes. If both the *CAN1* and *CYH2* genes on the plasmid are simultaneously inactivated by, for instance, a deletion mutation, the cells with the plasmid would become Can^R and Cyh^R. Therefore deletion mutations are preferentially detected rather than point mutation (Figure 1). A haploid *can1 cyh2* mutant, DH6.61D, was transformed with the plasmid YCpL2 and the Ura$^+$ transformants grown in liquid SD (omitting uracil) medium were plated on SD plates containing canavanine and cycloheximide. It was found that the cells resistant to both canavanine and cycloheximide appeared at a low but significant frequency. From the result of fluctuation analysis, the rate was 8.5×10^{-8}/cell/division cycle.

If the plasmid is unstable and plasmid-less strains appear frequently, it is possible that Ura$^+$ Can^R Cyh^R cells would be produced by both loss of the plasmid and reversion of the host *ura3* gene. We have studied the stability of the plasmid YCpL2 and showed that it is as stable as a common YCp vector (data not shown). We

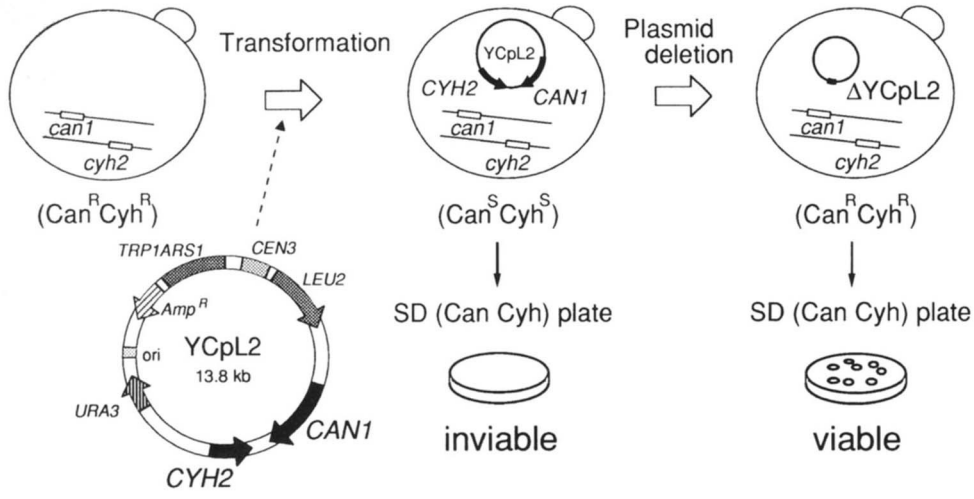

FIGURE 1.—System for detection of plasmid deletions. A plasmid YCpL2 carrying *CAN1* and *CYH2* genes was introduced into the haploid *can1 cyh2* strains. The transformants were sensitive to both canavanine (Can) and cycloheximide (Cyh). When a deletion covering *CAN1* and *CYH2* genes on YCpL2 occurs, the cells become resistant to both drugs. The rate of deletions on plasmid can be measured by plating YCpL2 transformants on SD plates containing canavanine and cycloheximide.

also confirmed that the growth rate of cells carrying deleted plasmid is not significantly different from that carrying intact YCpL2, because the ratio of Can^R Cyh^R cells carrying deleted plasmid to Can^S Cyh^S cells carrying intact YCpL2 was not significantly changed during 50 generations (data not shown).

When the plasmids were rescued from the Can^R Cyh^R cells, we could obtain recombinant plasmids from all of the Can^R Cyh^R cells (eight clones). The plasmids rescued from the Can^R Cyh^R cells were introduced into an *E. coli* strain and analyzed by agarose gel electrophoresis. All of eight rescued plasmids were found to be smaller than the parental plasmid YCpL2. Therefore, resistance to canavanine and cycloheximide appeared not to be due to plasmid loss coupled with Ura$^+$ reversion but more likely due to a deletion in the *CAN1-CYH2* region of the plasmid YCpL2. Restriction analysis of the eight recombinant plasmids showed that they have various sizes of deletions in the *CAN1-CYH2* region of the YCpL2 (Figure 2a). Since YCpL2 does not have any direct or inverted repeat of homologous sequences longer than 10 bp, it is suggested that the rearrangement is not attributable to homologous recombination but illegitimate recombination.

With regard to the eight YCpL2 recombinants analyzed by restriction mapping (Figure 2a), the recombination junctions were finely mapped by PCR and the nucleotide sequences at the recombination junctions were determined. The nucleotide sequences of the parental recombination sites were also determined. As shown in Figure 3a, there are short regions of homology (1–5 bp) at the recombination sites and no obvious feature at the junctions and their surroundings. The result also confirmed that the deletions were caused by illegitimate recombination.

Effects of *rad* mutations on the plasmid deletion: We

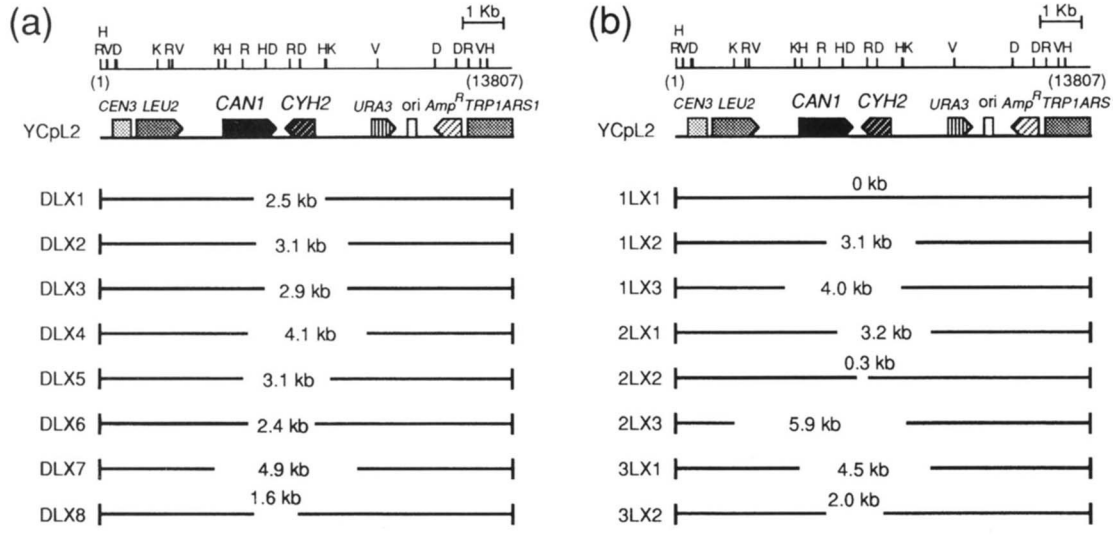

FIGURE 2.—Structural analysis of recombinant plasmids rescued from Can^R Cyh^R cells derived from *RAD*$^+$ strain and from *rad52* strain. Plasmids derived from Can^R Cyh^R cells were rescued in *E. coli recA* strains and analyzed by restriction mapping to determine the size and junction site of deletions. Sequences present in the plasmids are represented by a line. The numbers indicate the length of the deletion. *Eco*RI site between the *ARS1* and *CEN3* on YCpL2, whose length is 13,807 bp, is defined as position 1. Restriction sites shown are R, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; V, *Eco*RV; and D, *Dra*I. (a) *RAD*$^+$ strain. (b) *rad52* strain.

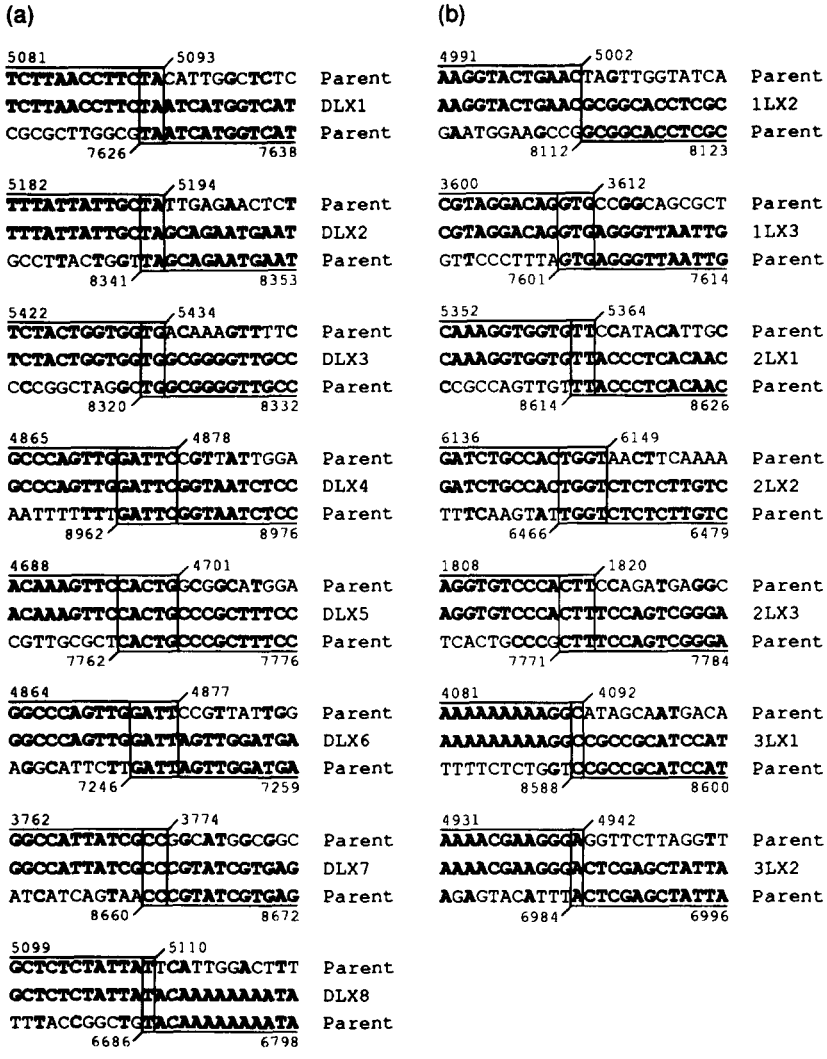

FIGURE 3.—Junction sequences of deleted plasmids derived from a *RAD*$^+$ strain and from a *rad52* strain. The top sequence represents the parental sequence corresponding to the left side of the junction, the middle sequence represents the sequence of the recombinant, and the bottom sequence represents the parental sequence corresponding to the right side of the junction. The site of the junction is represented by box. Homologous sequences around a junction are represented by bold letter. The orientation of DNA is 5′ to 3′ from left to right. Numbers represent the map coordinates of the YCpL2 sequence. (a) *RAD*$^+$ strain. (b) *rad52* strain.

have examined the effects of mutations of *RAD* genes, which are involved in double-strand break repair and homologous recombination, on the illegitimate recombination using this assay system. Each of the *RAD50, 51, 52, 54, 55, 57,* and *1, MRE11,* and *XRS2* genes of the strain DH6.61D was disrupted by one-step gene replacement and these disruptants were transformed with the plasmid YCpL2. The Ura$^+$ transformants grown in SD (omitting uracil) liquid medium were plated on SD plates containing both canavanine and cycloheximide. It was found that Can^R Cyh^R cells of the *rad52* mutant appeared at the rate of 2.7×10^{-9}/cell/division cycle, about one-thirtieth of the rate with parental *RAD52* strain DH6.61D, and Can^R Cyh^R cells of the *rad50, mre11,* and *xrs2* mutants appeared at the rate of 1.1×10^{-8}, 8.3×10^{-9}, and 9.9×10^{-9}/cell/division cycle, respectively, which were about one tenth of the rate of parental strain, while Can^R Cyh^R cells of the *rad51, rad54, rad55, rad57,* and *rad1* mutants appeared at the rate of 7.2×10^{-8}, 8.1×10^{-8}, 4.9×10^{-8}, 8.0×10^{-8}, and 5.3×10^{-8}/cell/division cycle, respectively, which were comparable with that of the parental strain (Figure 4). The result showed that most of the illegitimate recombinations detected with the plasmid YCpL2 are dependent on the Rad52, Rad50, Mre11, and Xrs2 functions but independent of Rad51, Rad54, Rad55, Rad57, and Rad1 functions.

The recombinant plasmids obtained from eight Can^R Cyh^R cells of the *rad52* mutant were rescued. Restriction analysis showed that seven of the eight plasmids have various sizes of deletions but one of them (1LX1 of Figure 2b) is indistinguishable from the parental plasmid YCpL2. We confirmed that the Can^R Cyh^R phenotype of cells carrying 1LX1 is caused by a mutation on plasmid by transforming DH6.61D again with the rescued 1LX1 plasmid DNA. The transformed DH6.61D cells showed resistance to both canavanine and cycloheximide. Therefore 1LX1 appears to have multiple point mutations or small rearrangements in both the *CAN1* and *CYH2* genes. The other seven recombinant plasmids were shown to have deletions. The recombination junctions were shown to be distributed randomly as shown in Figure 2b. The nucleotide sequences of the junctions showed that there are short regions of

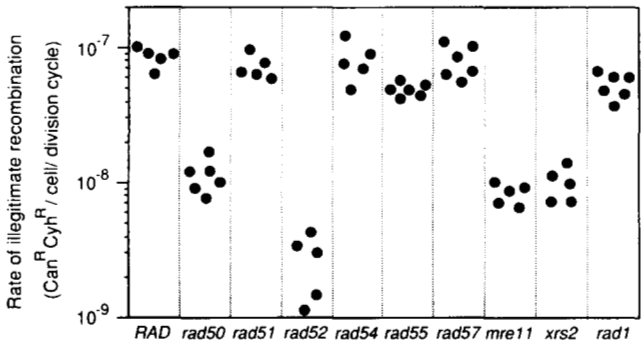

FIGURE 4.—The rate of plasmid deletions in *rad* mutants. Recombination rate was determined by fluctuation analysis and the data were analyzed by the methods of the median (LURIA and DELBRUCK 1943; LEA and COULSON 1948). The rate is represented as the number of deletion events per cell per division cycle. The rate of plasmid deletions and its deviation are described below. *rad:* average (standard deviation); *RAD:* 8.5 (1.3) $\times 10^{-8}$, *rad50:* 1.1 (0.3) $\times 10^{-8}$, *rad51:* 7.2 (1.4) $\times 10^{-8}$, *rad52:* 2.7 (1.2) $\times 10^{-9}$, *rad54:* 8.1 (2.4) $\times 10^{-8}$, *rad55:* 4.9 (0.5) $\times 10^{-8}$, *rad57:* 8.0 (2.0) $\times 10^{-8}$, *mre11:* 8.3 (1.2) $\times 10^{-9}$, *xrs2:* 9.9 (2.6) $\times 10^{-9}$, and *rad1:* 5.3 (1.0) $\times 10^{-8}$.

homology (1–4 bp) at the junctions of six recombinant plasmids (Figure 3b). With respect to one of the seven plasmids, 1LX2, there is no homologous region at the junction of that plasmid. One recombinant plasmid 2LX2 had a deletion of 330 bp and one of junction sites was in *CYH2* gene but the other was out of *CAN1* gene. DH6.61D transformed with 2LX2 showed resistance to both canavanine and cycloheximide. 2LX2 might have additional mutations in *CAN1* gene. From the sequence analyses of the recombination junctions in this work, the junction sequences of the Rad52-independent illegitimate recombination are not distinguishable from those of the Rad52-dependent illegitimate recombination.

DISCUSSION

We have developed a plasmid system for quantitative detection of illegitimate recombination. Using this system, we have investigated the effects of mutations of *RAD* genes, which are involved in double-strand break repair and homologous recombination (reviewed in PETES *et al.* 1991), on illegitimate recombination. The rate of illegitimate recombination was reduced by 30-fold in the *rad52* mutant, and 10-fold in *rad50, mre11,* and *xrs2* mutants, while it did not significantly change in the *rad51, rad54, rad55, rad57* and *rad1* mutants.

It has been known that *rad52* and *rad51* mutations reduce mitotic homologous recombination between genes with hetero-allele mutations, but *rad50, mre11,* and *xrs2* mutations do not affect this recombination (ALANI *et al.* 1990; MALONE *et al.* 1990; IVANOV *et al.* 1992; JOHZUKA and OGAWA 1995). Particularly, the Rad51 protein is also known to be a homologue of bacterial RecA protein and the Rad51 protein is physically associated with the Rad52 protein (ABOUSSEKHRA *et al.* 1992; BASILE *et al.* 1992; SHINOHARA *et al.* 1992; MILNE and WEAVER 1993). It was therefore thought that a complex of the Rad51 protein and the Rad52 protein plays a role in mitotic recombination (SHINOHARA *et al.* 1992; MILNE and WEAVER 1993). In contrast, the illegitimate recombination detected in our assay system is reduced by the *rad52* mutation, but not by the *rad51* mutation. This indicates that the Rad52 protein plays a role in illegitimate recombination independently of the Rad51 protein. This conclusion is in accord with the finding that mitotic homologous recombination between direct or inverted repeats of homologous sequences in a chromosome is reduced in a *rad52* mutant but increased or not remarkably decreased in a *rad51* mutant (MCDONALD and ROTHSTEIN 1994; RATTRAY and SYMINGTON 1994).

Structural analysis of the recombinant plasmids showed the presence of various sizes of deletions in the *CAN1-CYH2* region of YCpL2 and no hotspot of recombination was found in the wild-type strain and the *rad52* mutant. Sequence analysis showed that there was microhomology (1–5 bp) but not long region of homology at the junctions of recombinant plasmids rescued from the wild-type strain and *rad52* mutant. There was also no significant homology at the surroundings of recombination junctions. Therefore, the recombination that we detected was not homeologous recombination but illegitimate recombination. Because there is no long regions of homology at the recombination junction sites, it is unlikely that its DNA strand transfer activity and/or DNA annealing activity of the Rad52 protein (OGAWA *et al.* 1993a) play a role on illegitimate recombination.

In present study, the *rad52* mutation did not affect the length of homology at the recombination junctions. HABER and his coworker (KRAMER *et al.* 1994) also showed that imprecise rejoining of a double-strand break in *rad52* mutants requires short regions of homology (1–5 bp).

According to the double-strand break repair model involving homologous recombination, a double-strand break is followed by processing of DNA ends by an exonuclease(s) (Figure 5a) and strand invasion by Rad51 and related proteins (Figure 5b) (SUGAWARA *et al.* 1995). Rad52 protein may fulfill the function of homologous pairing in cooperation with Rad51 protein (SHINOHARA *et al.* 1992; MILNE and WEAVER 1993). In contrast, we propose an illegitimate recombination model that can explain possible roles of Rad52, Rad50, Mre11, and Xrs2 on the recombination as follows. Illegitimate recombination is probably initiated by a double-strand break of DNA, though the nature of the lesions that cause the double-strand break is not known. The broken ends may be processed by nuclease(s), thus producing a short single-stranded tail in DNA ends (Figure 5d). Finally they may be joined to form a viable

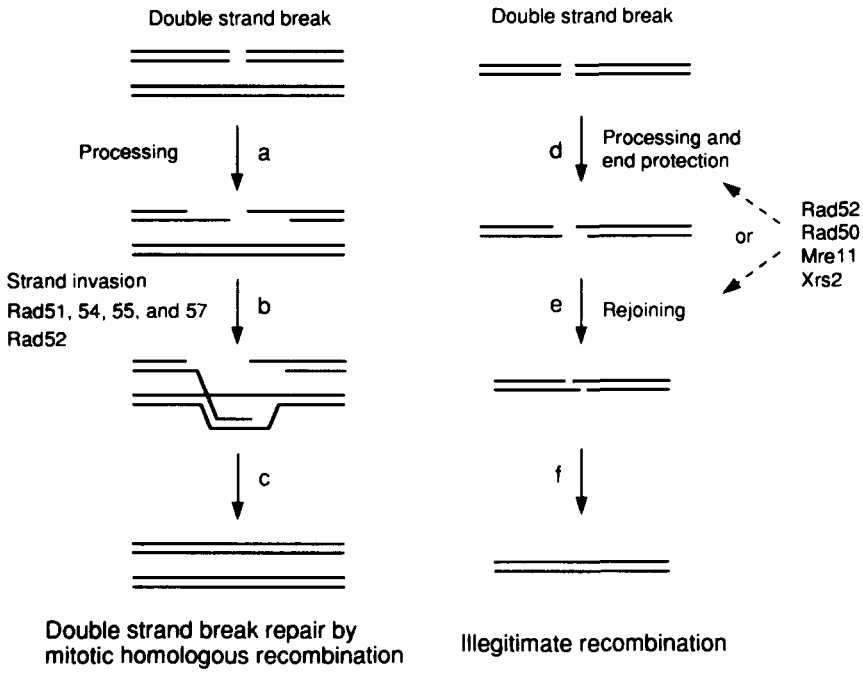

FIGURE 5.—Comparison of models for homologous recombination and illegitimate recombination. (a) In homologous recombination model proposed by SZOSTAK (SZOSTAK *et al.* 1983), each end of broken DNA is degraded by 5′ to 3′ exonuclease when double-strand break are occurred at one of the DNA molecule. The thick lines indicate homologous DNA molecules. (b) 3′ single-stranded ends invade into the homologous duplex DNA. Rad51, 52, 55, and 57 may be involved in this strand invasion step (SUGAWARA *et al.* 1995). (c) Following the formation of Holliday structure and its resolution, the double strand break of DNA is repaired effectively (ORR-WEAVER *et al.* 1981; ORR-WEAVER and SZOSTAK 1983). (d) In illegitimate recombination model, the ends of broken DNA are processed to form short single-stranded ends when double-strand break occurred on DNA molecule. (e) End-joining protein or other unknown protein rejoined with the ends of distant DNA molecule in a short-homology-dependent manner. (f) The gap is sealed by DNA ligase and/or DNA polymerase.

recombinant DNA (Figure 5, d–f). DNase activities associated with Rad52 or other Rad52-regulated functions may be involved in the formation of single-stranded ends in the course of illegitimate recombination (Figure 5d). Perhaps the Rad52 may have an activity for end joining similar to the kind that has been observed in mammalian cells (Figure 5e) (ROTH *et al.* 1985; ROTH and WILSON 1986; NORTH *et al.* 1990). It has been reported that the Rad52 protein did not have any nuclease activity, but the level of a DNase activity in a *rad52* mutant is less than one tenth of that in the wild-type strain (RESNICK *et al.* 1984; CHOW and RESNICK 1987, 1988). It has been also shown that the level of a DNase activity was higher in a *rad52* mutant than in the wild type strain (SUGAWARA and HABER 1992). In any cases, Rad52 protein may have multiple functions; it may have a function of homologous pairing together with Rad51 protein in homologous recombination and have a function of processing and/or end-joining in illegitimate recombination.

Because Rad50, Mre11 and Xrs2 proteins are physically associated with each other (JOHZUKA and OGAWA 1995) and the rate of illegitimate recombination was reduced by ~10-fold in these mutants, they may play a role in illegitimate recombination at the same step. It is known that *rad50, mre11,* and *xrs2* mutants are sensitive to X-ray irradiation and exhibit hyper-rec phenotype in mitotic homologous recombination (ALANI *et al.* 1990; MALONE *et al.* 1990; AJIMURA *et al.* 1992; IVANOV *et al.* 1992; JOHZUKA and OGAWA 1995). This indicates that Rad50, Mre11, and Xrs2 proteins may play a joint role in a double-strand break repair pathway that is not mediated by mitotic homologous recombination. In the double-strand break repair pathway, Rad50, Mre11, and Xrs2 proteins may conduct simple rejoining of broken DNA ends, while in illegitimate recombination these proteins may also perform a joining of two DNA ends by an activity such as control of nuclease activity or protection from nuclease attack (Figure 5, d and e).

It is also possible that the plasmid rearrangement may occur as a result of disordered homologous recombination between replicated plasmid molecules. For example, the sister chromatid exchange would give rise to dicentric dimer plasmid which is unstable and hence generates monocentric deleted plasmid. However, it is unclear whether the *rad52* mutation reduces this sister chromatid exchange or affect on stability of broken ends.

The effect of *rad* mutations on illegitimate recombination during integration are different from that of deletion mutations. SCHIESTL *et al.* (1994) showed that the frequency of illegitimate integration was reduced 100-, 8-, 20-, and 10-fold by the *rad50, 52, 51,* and *57* mutations, respectively. In the present study, the rate of plasmid deletion by illegitimate recombination was not reduced by the *rad51* and *57* mutations, but it was reduced 10- and 30-fold by the *rad50* and *52* mutations. These differences might suggest that the both recombination events occurred by different mechanisms or reflect different states of DNA molecule on chromosome and plasmid. SUGAWARA *et al.* (1995) showed that the requirement of the *RAD51, 54, 55,* and *57* genes for HO endonuclease-induced homologous recombination is different between plasmid and chromosome locus. They discussed that different states of DNA molecules might influence accessibility of single-strand tail at strand invasion step of homologous recombination. Then we have tried to confirm whether effects of *rad* mutations on illegitimate deletion on chromosome are similar to that on plasmid or not. YAMAGATA *et al.* (K. YAMAGATA, J. KATO and H. IKEDA, unpublished result)

have developed a system to detect chromosome deletions caused by illegitimate recombination using the modified YCpL2 plasmid. In this system, the *CAN1* and *CYH2* genes are inserted into the *LEU2* gene on chromosome *III* [*leu2::(CAN1 CYH2 HIS3)*] and the rate of deletion mutation in the *CAN1-CYH2* region was about 10^{-9}/cell/division cycle. The rate of deletion mutation on chromosome was remarkably low and hence it is impossible to examine the effects of *rad* mutations on illegitimate recombination in this system.

The plasmid YCpL2 made it possible to investigate, quantitatively, the plasmid rearrangement by illegitimate recombination. By use of the plasmid, we will be able to study the effects of mutations and to isolate the mutants, in which the illegitimate recombination is affected. A systematic analysis on these mutants will help to clarify details of the molecular mechanism of the illegitimate recombination.

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LITERATURE CITED

ABOUSSEKHRA, A., R. CHANET, A. ADJIRI and F. FABRE, 1992 Semidominant suppressors of Srs2 helicase mutations of *Saccharomyces cerevisiae* map in the *RAD51* gene, whose sequence predicts a protein with similarities to procaryotic RecA proteins. Mol. Cell. Biol. **12:** 3224–3234.

ADZUMA, K., T. OGAWA and H. OGAWA, 1984 Primary structure of the *RAD52* gene in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **4:** 2735–2744.

AJIMURA, M., S.-H. LEEM and H. OGAWA, 1992 Identification of new genes required for meiotic recombination in *Saccharomyces cerevisiae.* Genetics **133:** 51–66.

ALANI, E., L. CAO and N. KLECKNER, 1987 A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:** 541–545.

ALANI, E., S. SUBBIAH and N. KLECKNER, 1989 The yeast *RAD50* gene encodes a predicted 153-kD protein containing a purine nucleotide-binding domain and two large heptad-repeat regions. Genetics **122:** 47–57.

ALANI, E., R. PADMORE and N. KLECKNER, 1990 Analysis of wild-type and *rad50* mutants of yeast suggests an intimate relationship between meiotic chromosome synapsis and recombination. Cell **61:** 419–436.

ALBERTINI, A. M., M. HOFER, M. P. CALOS and J. H. MILLER, 1982 On the formation of spontaneous deletions: the importance of short sequences homologies in the generation of large deletions. Cell **29:** 319–328.

ALLGOOD, N. D., and T. J. SILHAVY, 1988 Illegitimate recombination in bacteria, pp. 309–330 in *Genetic Recombination,* edited by R. KUCHERLAPATI and G. R. SMITH. American Society for Microbiology, Washington, DC.

ALLGOOD, N. D., and T. J. SILHAVY, 1991 *Escherichia coli xonA (sbcB)* mutants enhance illegitimate recombination. Genetics **127:** 671–680.

BASILE, G., M. AKER and R. K. MORTIMER, 1992 Nucleotide sequence and transcriptional regulation of the yeast recombinational repair gene *RAD51.* Mol. Cell. Biol. **12:** 3235–3246.

BISHOP, D. K., D. PARK, L. XU and N. KLECKNER, 1992 *DMC1:* A meiosis-specific yeast homolog of *E. coli recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:** 439–456.

BROWN, P. A., and J. W. SZOSTAK, 1983 Yeast vectors with negative selection. Methods Enzymol. **101:** 278–290.

CHOW, T. Y.-K., and M. A. RESNICK, 1987 Purification and characterization of an endo-exonuclease from *Saccharomyces cerevisiae* that is influenced by the *RAD52* gene. J. Biol. Chem. **262:** 17659–17667.

CHOW, T. Y.-K., and M. A. RESNICK, 1988 An endo-exonuclease activity of yeast that requires a functional *RAD52* gene. Mol. Gen. Genet. **211:** 41–48.

COUKELL, M. B., and C. YANOFSKY, 1971 Influence of chromosome structure on the frequency of *tonB trp* deletions in *Escherichia coli.* J. Bacteriol. **105:** 864–872.

EHRLICH, S. D., 1989 Illegitimate recombination in bacteria, pp. 799–832 in *Mobile DNA,* edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, DC.

EKWALL, K., and T. RUUSALA, 1991 Budding yeast *CAN1* gene as a selection marker in fission yeast. Nucleic Acids Res. **19:** 1150.

FRANKLIN, N. C., 1967 Extraordinary recombinational events in *Escherichia coli.* Their independence of the *rec*$^+$ function. Genetics **55:** 699–707.

GRANT, S. G. N., J. JESSEE, F. R. BLOOM and D. HANAHAN, 1990 Differential plasmid rescue from transgenic mouse DNAs into *Escherichia coli* methylation-restriction mutants. Proc. Natl. Acad. Sci. USA **87:** 4645–4649.

IKEDA, H., H. SHIMIZU, T. UKITA and M. KUMAGAI, 1995 A novel assay for illegitimate recombination in *Escherichia coli:* stimulation of λ*bio* transducing phage formation by ultraviolet light and its independence from RecA function. Adv. Biophys. **31:** 197–208.

INSELBRUG, J., 1967 Formation of deletion mutations in recombination-deficient mutants of *Escherichia coli.* J. Bacteriol. **94:** 1266–1267.

ITO, H., Y. FUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkaline cations. J. Bacteriol. **153:** 163–168.

IVANOV, E. L., V. G. KOROLEV and F. FABRE, 1992 *XRS2,* a DNA repair gene of *Saccharomyces cerevisiae,* is needed for meiotic recombination. Genetics **132:** 651–664.

IVANOV, E. L., N. SUGAWARA, C. I. WHITE, F. FABRE and J. E. HABER, 1994 Mutations in *XRS2* and *RAD50* delay but do not prevent mating-type switching in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **14:** 3414–3425.

JOHZUKA, K., and H. OGAWA, 1995 Interaction of Mre11 and Rad50: Two proteins required for DNA repair and meiosis-specific double-strand break formation in *Saccharomyces cerevisiae.* Genetics **139:** 1521–1532.

KANS, J. A., and R. K. MORTIMER, 1991 Nucleotide sequence of the *RAD57* gene of *Saccharomyces cerevisiae.* Gene **105:** 139–140.

KLEIN, H. L., 1988 Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae.* Genetics **120:** 367–377.

KRAMER, K. M., J. A. BROCK, K. BLOOM, J. K. MOORE and J. E. HABER, 1994 Two different types of double-strand breaks in *Saccharomyces cerevisiae* are required by similar *RAD52*-independent, nonhomologous recombination events. Mol. Cell. Biol. **14:** 1293–1301.

KUMAGAI, M., and H. IKEDA, 1991 Molecular analysis of the recombination junctions of λ*bio* transducing phages. Mol. Gen. Genet. **230:** 60–64.

LEA, D. E., and C. A. COULSON, 1948 The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:** 264–284.

LOVETT, S. T., 1994 Sequence of the *RAD55* gene of *Saccharomyces cerevisiae:* similarity of RAD55 to prokaryotic RecA and other RecA-like proteins. Gene **142:** 103–106.

LOVETT, S. T., and R. K. MORTIMER, 1987 Characterization of null mutants of the *RAD55* gene of *Saccharomyces cerevisiae:* effects of temperature, osmotic strength and mating type. Genetics **116:** 547–553.

LURIA, S. E., and M. DELBRUCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MALONE, R. E., 1983 Multiple mutant analysis of recombination in yeast. Mol. Gen. Genet. **189:** 405–412.

MALONE, R. E., T. WARD, S. LIN and J. WARING, 1990 The *RAD50* gene, a member of the double strand break repair epistasis group, is not required for spontaneous mitotic recombination in yeast. Curr. Genet. **18:** 111–116.

MCDONALD, J. P., and R. ROTHSTEIN, 1994 Unrepaired hetero-

duplex DNA in *Saccharomyces cerevisiae* is decreased in *RAD1 RAD52*-independent recombination. Genetics **137:** 393–405.

MEUTH, M., 1989 Illegitimate recombination in mammalian cells, pp. 833–860 in *Mobile DNA,* edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, DC.

MILNE, G. T., and D. T. WEAVER, 1993 Dominant negative alleles of *RAD52* reveal a DNA repair/recombination complex including Rad51 and Rad52. Genes Dev. **7:** 1755–1765.

NAKAYAMA, N., A. MIYAJIMA and K. ARAI, 1985 Nucleotide sequences of *STE2* and *STE3,* cell type-specific sterile genes from *Saccharomyces cerevisiae.* EMBO J. **4:** 2643–2648.

NORTH, P., A. GANESH and J. THACKER, 1990 The rejoining of double-strand breaks in DNA by human cell extracts. Nucleic Acids Res. **18:** 6205–6210.

OGAWA, T., A. SHINOHARA, A. NABETANI, T. IKEYA, X. YU *et al.,* 1993a RecA-like recombination proteins in eukaryotes: functions and structures of *RAD51* genes. Cold Spring Harbor Symp. Quant. Biol. **58:** 567–576.

OGAWA, T., X. YU, A. SHINOHARA and E. H. EGELMAN, 1993b Similarity of the yeast RAD51 filament to the bacterial RecA filament. Science **259:** 1896–1899.

ORR-WEAVER, T. L., and J. W. SZOSTAK, 1983 Yeast recombination: the association between double-strand gap repair and crossing-over. Proc. Natl. Acad. Sci. USA **80:** 4417–4421.

ORR-WEAVER, T. L., J. W. SZOSTAK and R. J. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

PETES, T. D., R. E. MALONE and L. S. SYMINGTON, 1991 Recombination in yeast, pp. 407–521 in *The Molecular and Cellular Biology of the Yeast Saccharomyces. Genome Dynamics, Protein Synthesis and Energetics,* Vol. 1, edited by J. R. BROACH, J. R. PRINGLE and E. W. JONES. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

RATTRAY, A. J., and L. S. SYMINGTON, 1994 Use of a chromosomal inverted repeat to demonstrate that the *RAD51* and *RAD52* genes of *Saccharomyces cerevisiae* have different roles in mitotic recombination. Genetics **138:** 587–595.

RESNICK, M. A., A. SUGINO, J. NITISS and T. CHOW, 1984 DNA polymerases, deoxyribonucleases, and recombination during meiosis in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **4:** 2811–2817.

REYNOLDS, P., L. PRAKASH and S. PRAKASH, 1987 Nucleotide sequence and functional analysis of the *RAD1* gene of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **7:** 1012–1020.

ROSE, M. D., F. WINSTON and P. HIETER, 1990 *Methods in Yeast Genetics. A Laboratory Course Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ROTH, D. B., and J. H. WILSON, 1986 Nonhomologous recombination in mammalian cells: role for short sequence homologies in the joining reaction. Mol. Cell. Biol. **6:** 4295–4304.

ROTH, D. B., T. N. PORTER and J. H. WILSON, 1985 Mechanism of nonhomologous recombination in mammalian cells. Mol. Cell. Biol. **5:** 2599–2607.

ROTHSTEIN, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SAEKI, T., I. MACHIDA and S. NAKAI, 1980 Genetic control of diploid recovery after γ-irradiation in the yeast *Saccharomyces cerevisiae.* Mutat. Res. **73:** 251–265.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual,* Ed. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SCHIESTL, R. H., and T. D. PETES, 1991 Integration of DNA fragments by illegitimate recombination in *Saccharomyces cerevisiae.* Proc. Natl. Acad. Sci. USA **88:** 7585–7589.

SCHIESTL, R. H., and S. PRAKASH, 1988 *RAD1,* an excision repair gene of *Saccharomyces cerevisiae,* is also involved in recombination. Mol. Cell. Biol. **8:** 3619–3626.

SCHIESTL, R. H., M. DOMINSKA and T. D. PETES, 1993 Transformation of *Saccharomyces cerevisiae* with nonhomologous DNA: illegitimate integration of transforming DNA into yeast chromosomes and in vivo ligation of transforming DNA to mitochondrial DNA sequences. Mol. Cell. Biol. **13:** 2697–2705.

SCHIESTL, R. H., J. ZHU and T. D. PETES, 1994 Effect of mutations in genes affecting homologous recombination on restriction enzyme-mediated and illegitimate recombination in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **14:** 4493–4500.

SHIMIZU, H., H. YAMAGUCHI and H. IKEDA, 1995 Molecular analysis of λ*bio* transducing phage produced by oxolinic acid-induced illegitimate recombination *in vivo.* Genetics **140:** 889–896.

SHINOHARA, A., H. OGAWA and T. OGAWA, 1992 Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. Cell **69:** 457–470.

SUGAWARA, N., and J. E. HABER, 1992 Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. Mol. Cell. Biol. **12:** 563–575.

SUGAWARA, N., E. L. IVANOV, J. FISHMAN-LOBELL, B. L. RAY, X. WU *et al.,* 1995 DNA structure-dependent requirements for yeast *RAD* genes in gene conversion. Nature **373:** 84–86.

SUNG, P., 1994 Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. Science **265:** 1241–1243.

SZOSTAK, J. W., T. L. ORR-WEAVER and R. J. ROTHSTEIN, 1983 The double-strand-break repair model for recombination. Cell **33:** 25–35.

THOMAS, B. J., and R. ROTHSTEIN, 1989 The genetic control of direct-repeat recombination in *Saccharomyces:* the effect of *rad52* and *rad1* on mitotic recombination at *GAL10,* a transcriptionally regulated gene. Genetics **123:** 725–738.

VIEIRA, J., and J. MESSING, 1987 Production of single-stranded plasmid DNA. Meth. Enzymol. **153:** 3–11.

YAMAGUCHI, H., T. YAMASHITA, H. SHIMIZU and H. IKEDA, 1995 A hotspot of spontaneous and UV-induced illegitimate recombination during formation of λ*bio* transducing phage. Mol. Gen. Genet. (In press).

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