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A Unique Pathway of Double-Strand Break Repair Operates in Tandemly Repeated Genes

BRADLEY A. OZENBERGER and G. SHIRLEEN ROEDER*

Department of Biology, P.O. Box 6666, Yale University, New Haven, CT 06511-8112

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The *RAD52* gene product of the yeast *Saccharomyces cerevisiae* is required for most spontaneous recombination and almost all double-strand break (DSB) repair. In contrast to recombination elsewhere in the genome, recombination in the ribosomal DNA (rDNA) array is *RAD52* independent. To determine the fate of a DSB in the rDNA gene array, a cut site for the HO endonuclease was inserted into the rDNA in a strain containing an inducible *HO* gene. DSBs were efficiently repaired at this site, even in the absence of the *RAD52* gene product. Efficient *RAD52*-independent DSB repair was also observed at another tandem gene array, *CUP1*, consisting of 18 repeat units. However, in a smaller *CUP1* array, consisting of only three units, most DSBs (ca. 80%) were not repaired and resulted in cell death. All *RAD52*-independent DSB repair events examined resulted in the loss of one or more repeat units. We propose a model for DSB repair in repeated sequences involving the generation of single-stranded tails followed by reannealing.

Double-strand breaks (DSBs) are efficient initiators of homologous recombination in *Saccharomyces cerevisiae* (38, 56). Treatment of cells with X rays, which can generate DSBs, stimulates mitotic recombination more than 1,000-fold (38). Integration of linear DNA fragments during transformation is another example of recombination stimulated by DSBs (30). Other site-specific recombination events are also initiated by DSBs. For example, mating-type switching is initiated by a DSB introduced by the HO endonuclease at the *MAT* locus (51). It is not known what fraction of spontaneous mitotic recombination events are initiated by DSBs; however, double-strand gaps have been observed near sites of high-frequency meiotic gene conversion (6, 53).

An important factor in DSB repair is the product of the *RAD52* gene. *RAD52* was originally identified by a mutation that confers sensitivity to X rays (37). Evidence has since accumulated suggesting that a functional *RAD52* gene is required for almost all DSB repair in *S. cerevisiae*. Specifically, the *RAD52* gene product is essential for mating-type switching (25), for transformation with linear DNA (30), and for HO-induced DSB repair in duplicated sequences (28, 43). Spontaneous recombination is also greatly affected in a *rad52* mutant. *rad52* strains are defective in meiotic recombination and therefore fail to undergo proper meiotic chromosome segregation (13, 24, 34). Additionally, *rad52* strains are defective in most mitotic recombination, especially gene conversion (16, 17).

In contrast to events elsewhere in the genome, spontaneous recombination within the ribosomal DNA (rDNA) array occurs at wild-type levels in *rad52* strains (33, 60). The rDNA locus consists of a 9.1-kb sequence tandemly repeated approximately 100 times and represents about 8% of the yeast genome (3, 47). Recombination within this gene array has a number of unique properties. Meiotic interchromosomal exchange is greatly reduced (31, 32), a finding that may be related to the absence of synaptonemal complex within the nucleolus (5, 10). The *RAD50* gene, normally required for intrachromosomal meiotic recombination between duplicated sequences, is not essential for intrachromosomal events in the rDNA in meiosis (15). Further evidence of unusual recombinational properties of the rDNA is provided by the observations that mutations in the *TOP1*, *TOP2*, and *SIR2* genes stimulate mitotic intrachromosomal recombination within the rDNA array but have little or no effect on recombination at other loci (8, 14).

The absence of a requirement for the *RAD52* gene product for recombination in the rDNA array suggests either that recombination in the rDNA does not involve DSBs or that DSBs in yeast rDNA can be repaired by a *RAD52*-independent mechanism. To distinguish these possibilities, an HO endonuclease recognition site was inserted into the rDNA in a strain containing an inducible *HO* gene and a *rad52* gene disruption. HO-induced DSBs within the rDNA were efficiently repaired in the *rad52* mutant. The *RAD52*-independent DSB repair pathway is not unique to rDNA sequences but also operates in the much smaller *CUP1* gene array. *RAD52*-independent DSB repair events result in the loss of one or more repeat units, suggesting that this pathway requires the rejoining of DNA strands from different repeats with the concomitant loss of intervening sequences.

MATERIALS AND METHODS

Yeast strains and transformation. Yeast strains are listed in Table 1. Insertion of the HO cut site (HOcs) and *URA3* gene at the rDNA locus to create strain BOY15 is described below. Strain JH162 (provided by J. Haber) carries a $MAT\alpha_{inc}$ allele, which is not cleaved by the HO endonuclease (58), and an inducible *GAL-HO* gene created by the substitution of *GAL1-10* promoter sequences for the URS1 region conferring mother/daughter regulation of the *HO* gene (50). The *GAL-HO* gene is under normal cell type and cell cycle control but is transcriptionally induced only when cells are grown on galactose (50). Strain BOY57 was obtained from a cross between strains JH162 and BOY15. All other yeast strains described in this study are isogenic derivatives of BOY57. To develop a strain for the insertion of the HOcs and the *URA3* gene at the *CUP1* locus, a spontaneous Ura^- derivative of strain BOY57 (BOY68; produced by intrachromosomal recombination within the rDNA array) was selected on medium containing 5-fluoro-orotic acid (4). Strain

* Corresponding author.

TABLE 1. Strains[a]

Designation	Genotype
JH162	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3*
BOY15	*MAT***a** *his4-519 leu2-3,112 ura3-52 trpl-289 RDN::HOcs+URA3*
BOY54	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trpl-289 rad52::LEU2 RDN::HOcs+URA3*
BOY55	*MAT*α_{inc} *gal-ho::TRP1 his4 leu2-3,112 ura3-52 trpl-289 rad52::LEU2 RDN::HOcs+URA3*
BOY57	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trp1-289 RDN::HOcs+URA3*
BOY68	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trp1-289*
BOY103	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trp1-289 CUP1::HOcs+URA3(C)*
BOY131	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trp1-289 rad52::LEU2*
BOY140	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trp1-289 rad52::LEU2 CUP1::HOcs+URA3(C)*
BOY148	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trp1-289 rad52::LEU2 CUP1::HOcs+URA3(L)*
BOY174	*MAT*α_{inc} *GAL-HO his4 leu2-3,112 ura3-52 trp1-289 rad52::LEU2 CUP1(3)::HOcs+URA3*

[a] *HOcs+URA3* represents the insertion of HO cut site sequences and the *URA3* gene into the designated locus as described in the text and shown in Fig. 1. All strains except JH162 and BOY15 are isogenic. Strains BOY103 and BOY140 contain the *HOcs+URA3* insertion near the center of the 18-unit *CUP1* array. BOY148 contains the insertion near the left end of the array as described in the text. Strain BOY174 contains the *HOcs+URA3* insertion in the center unit of a three-unit *CUP1* array.

BOY131 is an HOcs⁻ Ura⁻ derivative of BOY54 (the *rad52::LEU2* derivative of BOY57), obtained by HO-induced DSB repair. Strain BOY174 contains only three *CUP1* repeats with an inserted HOcs and the *URA3* gene. An HO-induced recombinant of strain BOY140 that had lost 16 of 18 starting *CUP1* units was identified. This strain was then transformed with a replicating plasmid carrying the *RAD52* gene (YRp7-A4Sal-*RAD52*) (46) to facilitate subsequent insertion of a DNA fragment carrying the HOcs and the *URA3* gene at the *CUP1* locus.

Yeast strains were transformed by the lithium acetate procedure (48). Transformants were selected on synthetic complete (SC) medium lacking the appropriate nutrient (48).

Plasmids and DNA manipulations. Plasmid manipulations were performed by standard methods (26). Plasmids were maintained in *Escherichia coli* R895 (11).

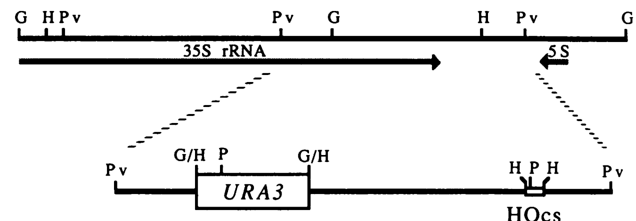
To disrupt the *GAL-HO* gene, a plasmid containing *HO* sequences interrupted by the *TRP1* gene was constructed. A 1.3-kb *Hin*dIII-*Bgl*II fragment containing a portion of the *HO* gene was isolated from pGAL-HO (18) and ligated into pUC18 restricted with *Hin*dIII and *Bam*HI. A 300-bp *Pst*I-*Bam*HI fragment near the center of the cloned *HO* DNA segment was then replaced with the 1.2-kb *Pst*I-*Bam*HI fragment carrying the *TRP1* gene from YRp7 (52). The final plasmid, designated pR998, was digested with *Kpn*I and *Pvu*II (which cleave only vector sequences) and introduced into *S. cerevisiae*.

To disrupt the *RAD52* gene, yeast cells were transformed with pSM20 (45) restricted with *Bam*HI. This construction introduces the *LEU2* gene into coding sequences near the 5′ end of the *RAD52* gene.

To introduce an HOcs in yeast rDNA, pOZ15 was constructed as follows. A 3.8-kb *Pvu*II fragment containing the 3′ end of the 35S rRNA gene and a portion of the spacer region was isolated from a cloned rDNA unit (constructed from cloned *Bgl*IIA and *Bgl*IIB fragments [20]) and used to replace the polylinker segment of pUC18. The HOcs was isolated on a 100-bp *Hin*dIII fragment from pAR134 (36) and inserted into the *Hin*dIII site of the cloned rDNA fragment (Fig. 1A). The yeast *URA3* gene was then inserted as a 1.1-kb *Hin*dIII fragment into the unique *Bgl*II site after filling in 5′ overhangs at the ends of both DNA fragments with the Klenow fragment of DNA polymerase I. The resultant plasmid, pOZ15 (Fig. 1A), was digested with *Pvu*II prior to transformation into yeast cells.

To insert the HOcs into the *CUP1* locus, a single *CUP1* unit was isolated as a 2.0-kb *Kpn*I fragment from pJW6 (12) and cloned into pUC19 restricted with *Kpn*I. Unique *Nco*I and *Eco*RV restriction sites were utilized as sites for the insertion of the HOcs fragment and the *URA3* gene, respectively (Fig. 1B). As described above, the HOcs and the

(A) rDNA



(B) *CUP1*

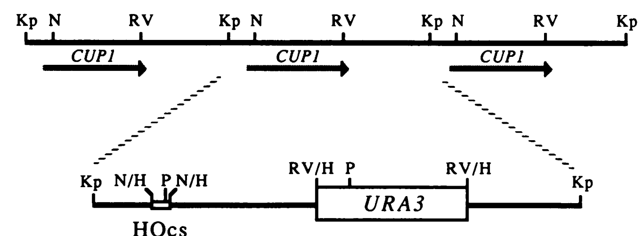


FIG. 1. DNA fragments used to insert the HOcs and the *URA3* gene into arrays. (A) Diagram of single rDNA unit showing the locations of the 35S and 5S rRNA genes. Shown below is the *Pvu*II fragment from pOZ15 used to insert an HOcs and the *URA3* gene into the rDNA array. The HOcs and the *URA3* gene are separated by 2,244 bp. (B) Diagram of three *CUP1* repeats. The approximate location of the major *CUP1* gene is shown. A second gene of unknown function is located adjacent to *CUP1* and is transcribed in the same direction (19). Shown below is the *Kpn*I restriction fragment from pOZ26 used to insert an HOcs and the *URA3* gene into the *CUP1* gene array. The HOcs and the *URA3* gene are separated by 897 bp. The *Kpn*I fragment represents a complete *CUP1* repeating unit. Restriction sites: H, *Hin*dIII; G, *Bgl*II; G/H, *Bgl*II-*Hin*dIII hybrid; Kp, *Kpn*I; N, *Nco*I; N/H, *Nco*I-*Hin*dIII hybrid; P, *Pst*I; Pv, *Pvu*II; RV, *Eco*RV; RV/H, *Eco*RV-*Hin*dIII hybrid.

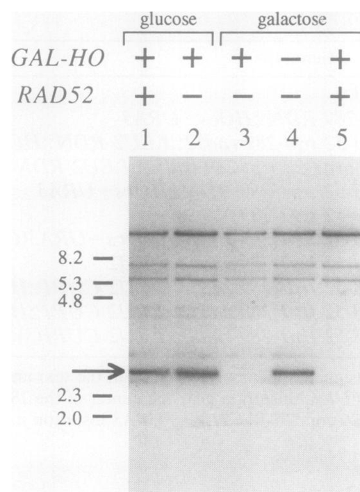


FIG. 2. Southern blot analysis of the HOcs fragment in the rDNA array. DNA was isolated from strains BOY54 (*GAL-HO rad52::LEU2 RDN::HOcs+URA3*) and BOY57 (*GAL-HO RAD52 RDN::HOcs+URA3*) grown on plates containing either glucose or galactose and strain BOY55 (*gal-ho::TRP1 rad52::LEU2 RDN::HOcs+URA3*) grown on a plate containing galactose as described in the text. DNA was digested with *Eco*RI, and the fragments were separated on an agarose gel and then blotted onto a nylon membrane. The blot was hybridized with a ^{32}P-labeled HOcs fragment. *RAD52* and *GAL-HO* genotypes and the growth medium are indicated above the autoradiogram. The rDNA fragment carrying the HOcs is 2.5 kb in size (indicated by an arrow). The autoradiogram shows slower-migrating *MAT*, *HML*, and *HMR* fragments that also hybridize to the HOcs probe. DNA markers are shown in the left margin in kilobases. Lanes: 1 and 5, BOY57; 2 and 3, BOY54; 4, BOY55.

URA3 gene were isolated on *Hin*dIII fragments, and protruding ends were filled in with Klenow fragment prior to ligation. The final plasmid, designated pOZ26, was cleaved with *Kpn*I prior to yeast transformation.

Recombination of a linear DNA fragment into tandemly repeated genes can occur by an "ends-out" mechanism resulting in a substitution event or by an "ends-in" alignment of homologous sequences resulting in integration of the fragment (56). In the construction of BOY174, integration into the two-unit *CUP1* array resulted in the formation of a three-unit array containing the HOcs and *URA3* gene in the center repeat.

Southern blot hybridization analysis. Correct insertion of linear DNA fragments following yeast transformations was verified by Southern blot analysis. Yeast DNA minipreps were performed as described by Sherman et al. (48). DNA blotting and hybridization was done on Zeta-probe nylon membrane, using the alkaline blotting procedure and hybridization conditions recommended by the supplier (Bio-Rad, Richmond, Calif.). Probes were labeled with ^{32}P by using a random oligonucleotide procedure (Prime-a-Gene; Promega Madison, Wis.).

To establish the presence or absence of the HOcs DNA fragment in the rDNA array, whole yeast DNA was digested with *Eco*RI and the fragments were separated in a 0.8% agarose gel. Following transfer to nylon membrane, the DNA blot was hybridized with the 100-bp HOcs fragment labeled with ^{32}P. The HOcs in the rDNA was present on a 2.5-kb *Eco*RI fragment (Fig. 2).

The loss of the HOcs during recombination in the *CUP1* array was established by observing the loss of a restriction site. DNA was isolated from derivatives of *CUP1::HOcs* strains and digested with *Pst*I, which cuts in the HOcs fragment and in the *URA3* gene. Southern blot hybridization analysis using *CUP1* sequences as the probe identified a 1.1-kb *Pst*I fragment diagnostic of the HOcs (Fig. 1B). Loss of the HOcs results in loss of the *Pst*I site and therefore disappearance of the 1.1-kb band.

To determine the number of *CUP1* units remaining after DSB repair in the 18-unit array, DNA was digested with *Eco*RI plus *Pst*I. Neither enzyme has recognition sites within *CUP1* DNA, but *Eco*RI cleaves unique sequences 1 to 2 kb beyond the ends of the array (19). The *Eco*RI fragment from Ura$^+$ recombinants is cut into two pieces by cleavage at the *Pst*I site in the *URA3* gene, generating DNA fragments no larger than 30 kb. To examine Ura$^-$ recombinants, it was necessary to fractionate the large *Eco*RI fragments (starting size equals 40 kb) by electrophoresis in a 40-cm-long 0.4% agarose gel run at 100 mA for 24 h in 1× Tris-borate buffer. DNA was blotted and hybridized with a *CUP1*-specific probe.

Pulsed-field gel electrophoresis. To determine the location of the inserted HOcs and *URA3* gene in the rDNA array in strain BOY57, pulsed-field gel electrophoresis was used to separate large DNA fragments. Intact chromosomes were isolated from yeast cells embedded in agarose beads by the following procedure. Yeast cells were grown overnight in 5 ml of YPD. Samples were harvested and resuspended in 3 ml of H_2O. After warming to 42°C, 4 ml of paraffin oil was added, followed by 0.75 ml 2.5% agarose maintained at 65°C. Samples were immediately vortexed with maximum splashing for at least 30 s and rapidly chilled in ice water while swirling. Then 5 ml of H_2O was added, the beads were pelleted, and H_2O and oil were removed. Beads were then washed in 10 ml of 1 M sorbitol–0.1 M sodium citrate–60 mM EDTA (pH 7)–60 mM 2-mercaptoethanol. Pellets were resuspended in the same buffer with the addition of zymolyase to 2.5 mg/100 ml and incubated at 37°C for 1 h. Beads were then pelleted and resuspended in 1% Sarkosyl–0.5 M Tris (pH 9)–0.2 M EDTA and incubated at 50°C for 30 min to lyse spheroplasts. Each sample was then washed six times in 10 ml of 1× restriction buffer with bovine serum albumin added to the last wash to a concentration of 0.1 mg/ml. Samples were digested with *Pst*I, which recognizes sites in the inserted HOcs and *URA3* gene but not within rDNA sequences. The resultant DNA fragments were separated in a 1% agarose gel by using a contour-clamped homogeneous electric field apparatus (9). Electrophoresis conditions were 200 V with a 90-s switching interval for 22 h at 10°C in 0.5× Tris-borate buffer. DNA was transferred to a nylon membrane by alkaline blotting as described above. The blot was hybridized to an rDNA-specific probe, and subsequent autoradiography revealed bands of approximately 450 and 500 kb, indicating that the insert was near the center of the array. This result also indicates that the rDNA array in the strains examined in these studies consists of approximately 100 copies of the 9.1-kb repeating unit.

Pedigree analysis. Strains to be examined by pedigree analysis were grown in medium containing 2% dextrose prior to induction. To release glucose repression, cells were washed once in water and then grown in YPL medium (1% yeast extract, 2% Bacto-Peptone [Difco], 3.5% lactic acid) for 16 h. A small quantity of each culture was placed on a YPGal plate consisting of the same ingredients as YPL with the addition of galactose to 2% (wt/vol). Single cells were then micromanipulated to isolated locations. As each cell

TABLE 2. Cell death in *rad52* strains

Generation	Dead cells/total cells (%)[a]			
	BOY54 *RDN::HOcs*	BOY140 *CUP1::HOcs(C)*	BOY148 *CUP1::HOcs(L)*	BOY174 *CUP1(3)::HOcs*
F_1	3/28 (10.7)	7/52 (13.5)	3/46 (6.5)	23/70 (32.9)[b]
F_2	4/50 (8.0)	13/88 (14.8)	14/86 (16.3)	34/90 (37.8)[b]
F_3	6/92 (6.5)	26/140 (18.6)[b]	20/134 (14.9)	46/102 (45.1)[b]
F_4	12/156 (7.7)	9/175 (5.1)	23/202 (11.4)	32/98 (32.7)[b]

[a] Values represent the number of cells that failed to produce daughters over the total number of cells examined at each generation. An isogenic *rad52* strain containing no HOcs (BOY131) produced dead cells at a frequency of 8.3% (78 dead of 940 examined) in pedigree analysis.
[b] Statistically different from the control strain BOY131 ($P < 0.05$).

budded, the daughter cell was transferred to another location. Each pedigree was monitored through four cell divisions (to the 16-cell stage). Colonies formed from fourth-generation cells on YPGal plates were examined for uracil auxotrophy by replica plating to SC-uracil medium. DNA was isolated from Ura^+ colonies and examined by Southern blot hybridization analysis, as described previously, to determine whether the HOcs was present. In addition, in the case of F_4 cells derived from strains carrying the HOcs in the rDNA, the possibility that the HOcs might be present in a small fraction of Ura^+ cells was explored as follows. Colonies derived from F_4 cells (grown on YPGal plates) were streaked for single colonies on YPD plates (48). Several isolated colonies from each sample were then patched onto YPD and replica plated to SC-uracil. Ura^+ $HOcs^-$ recombinants produced almost exclusively Ura^+ cells in this test, while samples that were still $HOcs^+$ produced predominantly Ura^- cells due to continued expression of *GAL-HO*. Contingency chi-square tests (49) were used to determine the statistical significance of cell death frequencies.

Determination of ploidy. The *rad52* strains used in these studies spontaneously became diploid. This made it necessary to examine *rad52* strains for ploidy both before and after pedigree analyses. Cells from isolated colonies were mated to a known haploid *MAT***a** strain. The resulting diploids (or triploids) were then sporulated, and the viability of spores was determined by exposure to ether (41). Diploid strains demonstrated good spore viability. Triploids, however, produced almost no viable spores. Samples shown to be haploid in this manner were subsequently used for pedigree analysis. Because of expansion of the starting culture prior to pedigree analysis, it was also necessary to determine the ploidy of each sample after analysis. The ploidy of one colony from each pedigree was checked. All results presented below were derived from haploid cells.

RESULTS

HO-induced DSBs in the rDNA array are repaired in a *rad52* mutant. To examine *RAD52*-independent repair of a DSB in yeast rDNA, a strain was constructed with an inducible *HO* gene and an HO nuclease recognition sequence (HOcs) in the rDNA. The initial strain, BOY57 (Table 1), contains a $MAT\alpha_{inc}$ allele that is not cleaved by the HO endonuclease and a *GAL-HO* gene that is induced by growth on galactose. In addition, BOY57 contains an HOcs and a *URA3* gene inserted into the rDNA array in the configuration shown in Fig. 1A. Pulsed-field gel electrophoresis followed by Southern blot analysis demonstrated that the inserted sequences are near the center of the array (see Materials and Methods). *rad52::LEU2* and *rad52::LEU2 gal-ho::TRP1* derivatives of BOY57 were constructed by one-step gene disruption (42) as described in Materials and Methods. These strains were designated BOY54 and BOY55, respectively (Table 1).

The three strains, BOY57, BOY54, and BOY55, were derepressed in medium containing lactic acid (YPL) and then plated for single colonies on medium containing either glucose, to repress *GAL-HO* expression, or galactose, to induce *GAL-HO*. Repair of an HO-induced DSB in the rDNA by recombination with a repeat unit lacking the HOcs results in loss of the HOcs sequences. DNA extracted from isolated colonies was examined by Southern blot hybridization using HOcs sequences as a probe to determine whether the HOcs had been removed (Fig. 2). The HOcs was absent from the *GAL-HO RAD52* and *GAL-HO rad52::LEU2* strains grown on medium containing galactose but retained in the *ho::TRP1* derivative and in strains grown on medium containing glucose. Although most DNA samples isolated from cells expressing *GAL-HO* showed no band of the size representing the HOcs in the rDNA, a few samples did exhibit weak signals, suggesting that not all cells within a given colony had experienced HO cleavage (data not shown). This finding suggests that cleavage of the HOcs in the rDNA is not 100% efficient.

To determine whether loss of the HOcs in the rDNA is truly a result of double-strand break (DSB) repair, DNA was isolated from derepressed BOY57 cells harvested at various times after the addition of galactose. Samples were digested with *Bgl*II and examined by Southern blot analysis, using an *URA3*-specific probe, for the appearance of an HO-induced DSB in the rDNA. The *URA3* gene is present on a 10.3-kb *Bgl*II fragment which should be truncated to 8.0 kb when cleaved by the HO nuclease. The 8.0-kb *Bgl*II-HO fragment was clearly evident at 1 h postinduction and remained visible until 7 h (data not shown).

Examination of DSB repair by pedigree analysis. To investigate the efficiency of DSB repair in the rDNA, single cells were examined by pedigree analysis as described in Materials and Methods. Derepressed cells were micromanipulated on YPGal medium through four generations to examine the frequency of DSB-induced cell death and to obtain DSB-induced recombinants for further analysis. Following the fourth division (producing a maximum of 16 cells from each starting cell), surviving cells were allowed to form colonies on the YPGal plate. Colonies were examined for uracil auxotrophy, and Ura^+ colonies were examined for loss of the HOcs as described in Materials and Methods. Ura^- colonies were assumed to have lost the HOcs.

The frequencies of cell death observed at each generation during pedigree analysis of the *RDN::HOcs rad52* strain (BOY54; Table 2) were not significantly different from those observed in an isogenic *rad52* strain lacking an HOcs

TABLE 3. HO-induced recombination in gene arrays

Strain	Genotype	No. (%)[a]	
		Ura⁻	Ura⁺
BOY54	*RDN::HOcs rad52*	64 (79)	17 (21)
BOY57	*RDN::HOcs RAD52*	36 (36)	64 (64)
BOY140	*CUP1::HOcs(C) rad52*	162 (98)	4 (2)
BOY148	*CUP1::HOcs(L) rad52*	155 (87)	24 (13)
BOY103	*CUP1::HOcs(C) RAD52*	70 (36)	122 (64)

[a] Total number of HOcs⁻ survivors of each recombinant class following pedigree analyses and the percentage of cells in each class. Cells that retained the HOcs after four generations were not included in calculations. The frequency of Ura⁻ recombinants in cultures prior to induction was <1%; no Ura⁻ cells were included in the pedigree analyses. The minimum number of independent recombination events for each strain was as follows: BOY54, 44; BOY57, 47; BOY140, 54; BOY148, 54; BOY103, 59.

(BOY131; frequency of cell death on YPGal medium equals 8.3%). Cell death was negligible (<0.5%) in the *RAD52* strain (BOY57). The lack of a significant increase in cell death after *GAL-HO* induction suggests that the efficiency of DSB repair is very high in the rDNA array regardless of the presence or absence of the *RAD52* gene product.

A significant percentage of F_4 cells from both the *rad52* and *RAD52* strains (39 and 19%, respectively) had not yet experienced DSBs, as shown by the retention of the HOcs. These samples were excluded from further analysis. Products of HO-induced recombination in the rDNA were of two classes, Ura⁻ or Ura⁺. Recombination in the *rad52* strain (BOY54) usually resulted in the loss of the *URA3* gene (79% of recombinants), but many recombinants (21%) were Ura⁺ (Table 3). In the *RAD52* strain (BOY57), only 36% of the recombinants were Ura⁻ (Table 3). These results suggest that the *RAD52* gene product plays a role in DSB repair in the rDNA.

***RAD52*-independent DSB repair at the *CUP1* locus.** To determine whether *RAD52*-independent DSB repair is unique to the rDNA, experiments similar to those done with the rDNA array were performed at the *CUP1* locus, another tandem gene array. In the initial strains used for these studies, the *CUP1* array consists of 18 copies of a tandemly repeated 2.0-kb unit (19).

The HOcs fragment and the *URA3* gene were inserted into the *CUP1* array by transformation of strain BOY68 with the DNA fragment shown in Fig. 1B. The locations of inserts within the *CUP1* array were determined by Southern blot analysis. Strains BOY103 and BOY140 carry the HOcs and the *URA3* gene in the ninth unit from the left end of the *CUP1* array (as drawn in Fig. 1B), whereas strain BOY148 carries the insert in the third unit from the left. In the genotypes of these strains, the position of the HOcs within the *CUP1* array is indicated by a *C* (for center) or *L* (for left end). Note that these strains are isogenic to the *RDN::HOcs* strains, differing only in the location of the HOcs and the *URA3* gene.

RAD52-independent DSB repair was observed in the *CUP1* array (Table 3). Most recombination events in the *rad52 CUP1::HOcs+URA3* strains produced Ura⁻ cells (BOY140, 98%; BOY148, 87%). In the *RAD52* strain (BOY103), fewer recombinants were Ura⁻ (36%; Table 3). Cleavage of the HOcs inserted into *CUP1* was found to be more efficient than in the rDNA. In the *RDN::HOcs* strains, 19 to 39% of F_4 cells retained the HOcs; in *CUP1::HOcs* strains, however, no cells retained the HOcs after pedigree analysis. Although the cutting efficiency was evidently greater at the *CUP1* locus, the timing of DSB formation was similar to that observed in the rDNA (data not shown).

TABLE 4. Loss of units at *CUP1*

No. of units lost	Recombinants[a]								
	BOY140 *CUP1::HOcs(C) rad52*			BOY148 *CUP1::HOcs(L) rad52*			BOY103 *CUP1::HOcs(C) RAD52*		
	Ura⁻	Ura⁺	%[b]	Ura⁻	Ura⁺	%	Ura⁻	Ura⁺	%
0	0	0		0	0		9	5	32
1	5	0	10	6	2	19	7	20	61
2	6	2	16	10	4	33	0	0	
3	4	1	10	5	0	12	0	0	
4	6	0	12	6	0	14	0	0	
5	5	1	12	4	0	9	1	0	2
6	4	0	8	0	0		0	0	
7	10	0	20	1	0	2	0	0	
8	1	0	2	0	0		1	1	5
9	0	0		2	0	5	0	0	
10	3	0	6	2	0	5	0	0	
11	2	0	4	1	0	2	0	0	
16	1	0	2	0	0		0	0	

[a] The number of independent events examined for each strain was 51 for BOY140, 43 for BOY148, and 44 for BOY103.

[b] Percentage of total recombinants examined that lost the indicated number of *CUP1* units. All DNA samples (each derived from a single F_4 cell colony) contained a *CUP1* array of only one size, confirming that the events occurred at or prior to the F_4 generation.

In the *rad52 CUP1::HOcs* strains, there was a slight increase in the frequency of cell death relative to an isogenic *rad52* control strain lacking an HOcs (strains BOY140 and BOY148; Table 2). It is important to note that the frequencies of cell death reported in Table 2 underestimate the frequency of nonrepaired DSBs because the total cell population at every generation (except F_1) includes cells that have already lost the HOcs. The cell death frequencies observed for BOY140 and BOY148 for the F_2 and F_3 generations were higher than for the HOcs⁻ control, but only the increase in the frequency of cell death in the F_3 generation of strain BOY140 (18.6%, Table 2) was statistically significant ($P < 0.05$). The frequencies of cell death in the *CUP1::HOcs* strains decreased in the F_4 generation (BOY140 and BOY148; Table 2) probably because the HOcs sequences had already been removed by DSB repair in most cells. These results suggest that *RAD52*-independent DSB repair is less than 100% efficient in the 18-unit *CUP1* array.

The small size of the *CUP1* array (36 kb) compared with the rDNA array (950 kb) permitted the detection of changes in repeat copy number following DSB-induced recombination. DNA samples isolated from recombinant strains following pedigree analysis were examined by Southern blot hybridization to determine the size of the array. All of the recombinants isolated from the *rad52* strains lost units (Table 4). DSB repair in these stains resulted in the loss of 1 to 16 of the 18 starting units, with medians of 5 *CUP1* units lost in the *CUP1::HOcs(C)* strain (BOY140) and 2 units lost in the *CUP1::HOcs(L)* strain (BOY148). The smaller number of units lost with the HOcs located near an end of the array may be due to a constraint in the repair process introduced by the proximity of unique sequences. This limitation was most apparent when the Ura⁺ recombinants were examined. In the *CUP1::HOcs(L)* strain (BOY148), there are only two *CUP1* repeats between the *URA3* gene and the end of the array, so a maximum of two units could be lost for a recombinant to remain Ura⁺. Of the six Ura⁺ recombinants

TABLE 5. DSB-induced events in a three-unit *CUP1* array[a]

Generation	No. of cells[b]	No. of events[c]	No. dead[d] (%)	No. Ura⁻ (%)	No. Ura⁺ (%)
F_1	70	27	23	2	2
F_2	86	48	34	11	3
F_3	72	57	46	10	1
F_4	36	36	32	3	1
Total	264	168	135 (80)	26 (15)	7 (4)

[a] Numbers are derived from 70 pedigree analyses of strain BOY174. The individual cells within a pedigree that experienced DSBs were deduced from the phenotypes of the F_4 survivors as explained in the text.

[b] Total number of cells that could potentially experience a DSB. Progeny of $HOcs^-$ recombinants were not included in these values.

[c] Deduced number of DSB events resulting in either death or recombination.

[d] No correction was made for random cell death, which occurs at a frequency of 8.3%.

examined in this strain, each had lost only one or two units (Table 4). In sharp contrast to repair events in the *rad52* strains, most DSB-induced recombination events in strain BOY103 (*RAD52*) resulted in the loss of one or no units (Table 4).

***RAD52*-independent DSB repair in a three-unit *CUP1* array.** To determine whether repair efficiency might be correlated with array size, a *rad52* strain with only three *CUP1* repeats was constructed. One HO-induced recombinant from strain BOY140 had lost 16 of 18 *CUP1* repeats (Table 4). The HOcs and the *URA3* gene were integrated into this recombinant as described in Materials and Methods to create the *CUP1 (3)::HOcs+URA3* strain, BOY174. This strain has three *CUP1* repeats with the HOcs and the *URA3* gene located in the center unit.

Pedigree analysis of the three-unit strain revealed that *RAD52*-independent DSB repair was inefficient, with many DSBs resulting in cell death (BOY174; Table 2). The percentage of dead cells per generation was as high as 45.1 (Table 2). Because of the high frequency of cell death and the low frequency of recombination in this strain, it was possible to assign recombination events to specific cells in each pedigree with some confidence. For example, if all four granddaughter cells derived from a single F_2 cell were Ura^-, it was assumed that these were the products of a single recombination event that occurred in the F_2 cell. The summation of all events from 70 pedigrees of the *CUP1 (3)::HOcs+URA3 rad52* strain (BOY174) is presented in Table 5. By examining events rather than total cells, it is clear that the *RAD52*-independent DSB repair efficiency is very low (80% cell death). Of the DSB-induced recombination events, those which lost the *URA3* gene occurred almost four times more frequently than those which did not (26 Ura^-, 7 Ura^+; Table 5).

All repair events in the *CUP1(3)::HOcs+URA3 rad52* strain (BOY174) resulted in the loss of DNA. Of the 26 Ura^- recombinants, 10 had lost one repeat and 16 had lost two of the three starting *CUP1* units. Each of the Ura^+ recombinants had lost one repeat. These data, together with the data obtained from the 18-unit *rad52* strains (Table 4), clearly indicate that *RAD52*-independent DSB repair in a gene array involves the loss of repeats. Of 127 independent repair events examined (Tables 4 and 5), all had lost at least one *CUP1* repeat.

DISCUSSION

DSBs in tandemly repeated sequences can be efficiently repaired in *rad52* strains. Previous studies have suggested that the *RAD52* gene product is essential for DSB repair (25, 28, 30, 40, 43, 57). However, we have observed efficient *RAD52*-independent DSB repair in the rDNA and *CUP1* gene arrays (Tables 2 and 3), demonstrating that DSB repair in tandemly repeated sequences does not require the *RAD52* gene product. Pedigree analysis of *rad52* strains containing unique HOcs sequences in the rDNA array or 18-unit *CUP1* array revealed high efficiencies of DSB repair upon induction of *GAL-HO*, with little or no cell death (Table 2). However, in a *rad52* strain carrying the HOcs in a three-unit *CUP1* array, the increase in the frequency of cell death upon induction of *GAL-HO* was dramatic (BOY174; Tables 2 and 5). Thus, *RAD52*-independent DSB repair is efficient in large gene arrays, but the efficiency is reduced in an array containing fewer repeats.

Recombination events in *RAD52* strains produced different ratios of recombinant classes compared with events in the *rad52* strains. The *URA3* gene is located near the HOcs in each array (2,244 bp away in rDNA; 897 bp away in *CUP1*) and was lost more frequently in *RAD52*-independent DSB repair events (Table 3). Previous studies have demonstrated that spontaneous sister chromatid exchange events in the rDNA array are not dependent on the *RAD52* gene product (33, 60). However, the results presented here indicate that the *RAD52* gene can be involved in recombination in the rDNA. Additionally, we have shown that the absence of a strict requirement for *RAD52* does not a priori preclude recombination pathways initiated by DSBs.

***RAD52*-independent DSB repair results in the loss of repeat units.** Following pedigree analysis of the *CUP1::HOcs* strains, recombinants were examined for changes in repeat copy number. DSB repair in the *rad52* mutants always resulted in the loss of units (Table 4). As many as 16 units were lost from an 18-unit array, with median losses of two or five repeats, depending on the location of the HOcs. Most DSB repair events in the Rad^+ *CUP1::HOcs* strain resulted in the loss of DNA as well (BOY103; Table 4). However, only 7% of recombinants had lost more than one repeat, and the primary recombinant class in the Rad^+ strain had lost one unit (61%; Table 4). The dissimilarities in DSB repair products in *rad52* and *RAD52* strains emphasize differences in DSB-induced recombination pathways associated with the presence or absence of the *RAD52* gene product.

Several investigators have examined spontaneous recombination between duplicated sequences in *rad52* mutant strains. Klein (21) examined prototrophic recombinants generated from a *his3* duplication in *rad52-1* mutant strains. She observed that 91 to 98% of recombinants (excluding G2 triplication events) resulted from pop-outs (resulting in loss of one of the repeat units and intervening sequences) and that the remainder resulted from gene conversion. Jackson and Fink (17) examined spontaneous mitotic recombination between *his4* repeats in a *rad52-1* strain and found that 86 to 97% of His^+ recombinants involved plasmid excision. In both laboratories, overall frequencies of His^+ prototrophy were reduced at least 10-fold in the *rad52* mutant compared with the wild type, and gene conversion events were reduced 200- to 300-fold. However, the rates of plasmid excision were only slightly affected by the *rad52* mutation. Comparison of these results with those presented here suggests that DSB-induced recombination events in *rad52* strains are mechanistically similar to spontaneous events, as both path-

ways generally involve the loss of at least one repeat and intervening sequences.

Mechanisms of DSB repair in reiterated sequences. Simple gap repair is a predominant mechanism of DSB repair in repeated sequences in Rad$^+$ cells (28, 36). DSB repair frequently involves the formation of extensive regions of heteroduplex DNA in sequences flanking the break (16, 28, 29, 35, 36). The frequency with which a marker near a DSB undergoes gene conversion, whether by gap expansion or heteroduplex formation followed by mismatch repair (see reference 54), is directly correlated to the distance of the marker from the DSB (28, 29, 36).

About one-third of the recombination events in the *RAD52 CUP1*::*HOcs* strain (BOY103) did not result in the loss of repeats and therefore likely resulted from gap repair. Repair by this mechanism requires that DSB formation be followed by exonucleolytic digestion to remove at least the HOcs sequences, thereby generating ends homologous to *CUP1*. In comparable experiments, Nickoloff et al. (28) found that one-fifth of DSB repair events at a *ura3* duplication occurred via gap repair without the loss of intervening sequences. Of those events, 40% demonstrated coconversion of a site 200 bp from the DSB. The extent of gap expansion or heteroduplex formation at *CUP1* must often be considerable since many recombinants that lost no units were Ura$^-$ (9 of 14; Table 4), indicating inclusion of the *URA3* gene in the conversion tract (897 bp from the HOcs). The DSB repair model (54) can also account for those recombinants at *CUP1* that lost one or more units if gene conversion is associated with reciprocal crossing over or if the initial gap is larger than one repeat. Events involving unit loss can also be explained by a single-strand chew-back, reanneal, and repair model, as described below.

Strand recision following DSB formation may be an important step in DSB-induced recombination in *S. cerevisiae* (1, 6, 28, 29, 35, 36, 39, 53, 54, 56). Exonucleolytic digestion in a 5′-to-3′ direction has been demonstrated following HO cleavage at *MAT* (59). Lin et al. (22) were the first to propose the formation of single-strand tails at DSBs as an important step in recombination between repeated sequences. Several reports of intra- and intermolecular recombination-repair events that apparently involve extensive exonuclease activity in mammalian cells (2, 7, 23) and the demonstration of extensive 5′-to-3′ exonuclease activity in *Xenopus laevis* oocyte nuclei (27) support this model.

We suggest that after DSB formation at *CUP1* or in the rDNA, strands are chewed back preferentially in one direction (5′ to 3′), exposing complementary single-stranded sequences (Fig. 3). Strands could then reanneal with each other, 3′ tails could be removed, and single-strand gaps could be filled in to complete repair. Such a pathway would always result in the loss of at least one repeat. In the 18-unit *CUP1 rad52* strains, DSB repair usually resulted in the loss of several units (Table 4), suggesting that strand recision can frequently involve more than one repeat. 5′-to-3′ exonucleolytic activity has been reported to be more extensive in *rad52* mutants than in wild-type cells (59), perhaps accounting for the greater number of units lost in the *rad52 CUP1*::*HOcs* strains than in the *RAD52 CUP1*::*HOcs* strain (Table 4).

Most *RAD52*-independent DSB repair events resulted in the loss of the *URA3* gene. There are several means of explaining this result in terms of the recision-reannealing model. With one strand of the *URA3* gene removed, the *URA3* sequences in the other strand would have no complement upon reannealing. This looped-out region might subsequently be removed by mismatch correction. If mismatch repair is the primary mechanism, then the direction of repair must be strongly biased, usually resulting in the removal of unmatched *URA3* sequences rather than resynthesis of the complementary DNA strand. Alternatively, reannealing might not involve the repeat unit containing *URA3* but rather one further from the DSB. In this case, *URA3* would be included in a single-strand tail that would be subsequently removed by nucleolytic activity (Fig. 3). A third possibility is that exonucleolytic digestion of both strands precedes the reannealing step, but 5′-to-3′ recision is much more extensive than digestion in the 3′-to-5′ direction. In the case of the *CUP1* array, recision in the 3′-to-5′ direction would have to extend less than 900 bp before the *URA3* gene would no longer be flanked (on both sides) by *CUP1* sequences and therefore would be unable to generate an insertion loop upon reannealing.

The data presented here indicate that the efficiency of *RAD52*-independent DSB repair decreases as the number of repeats in the array decreases. Digestion of both DNA strands beyond the *CUP1* sequences would prevent repair of the DSB by homologous recombination and result in cell death. In the three-unit *CUP1* strain (BOY174, 80% cell death; Table 5), the HOcs is only 2.3 kb from unique sequences. Thus, these data suggest that recision of both strands may be extensive in *rad52* strains. Alternatively, the high frequency of cell death in the three-unit *CUP1* strain could be due to inefficient annealing of complementary strands that contain only a single intact repeat.

The recision-reannealing pathway of DSB repair in directly repeated genes may occur frequently in Rad$^+$ cells but is greatly emphasized in *rad52* strains. The predominant class of recombinants in the *RAD52 CUP1*::*HOcs* strain (BOY103; Table 4) had lost one repeat, consistent with a recision-reannealing pathway (with less extensive strand recision compared with the *rad52* strains). Rudin et al. (44) examined DSB-induced recombination between directly repeated *LACZ* sequences on a plasmid and on a chromosome in Rad$^+$ strains and found that the majority of events (83% in the chromosomal assay and 67% in the plasmid assay) resulted in the loss of one repeat and intervening sequences. Attempts were made to physically detect the reciprocal product of recombination, which is expected to be a closed circular molecule if the deletion is the result of a reciprocal crossover. No circles were detected, providing evidence for a nonreciprocal mechanism such as recision-reannealing.

Other mechanisms of DSB repair are consistent with the data presented. One pathway involves end invasion with the direct participation of sequences to only one side of the DSB. Following HO cleavage and limited gap expansion to remove HOcs sequences, one end might find homology in another repeat unit and the duplexes could then be rejoined with the loss of intervening sequences. This mechanism is similar to the λ Red system (56), in which a DSB initiates the joining of one end of a gap to another homologous segment of DNA. A one-end invasion pathway predicts that equal numbers of Ura$^-$ and Ura$^+$ recombinants should be observed if the two sides of the DSB are equivalent. However, the difference in the frequencies of Ura$^-$ and Ura$^+$ recombinants in *RAD52*-independent DSB repair events is large (79 to 98% Ura$^-$; Table 3). To account for the high frequencies of Ura$^-$ recombinants in a one-end invasion pathway, *RAD52*-independent DSB repair must involve either frequent expansion of the gap beyond the *URA3* gene or directionality to repair events such that the end that is not attached to *URA3* is more frequently the invading end. In

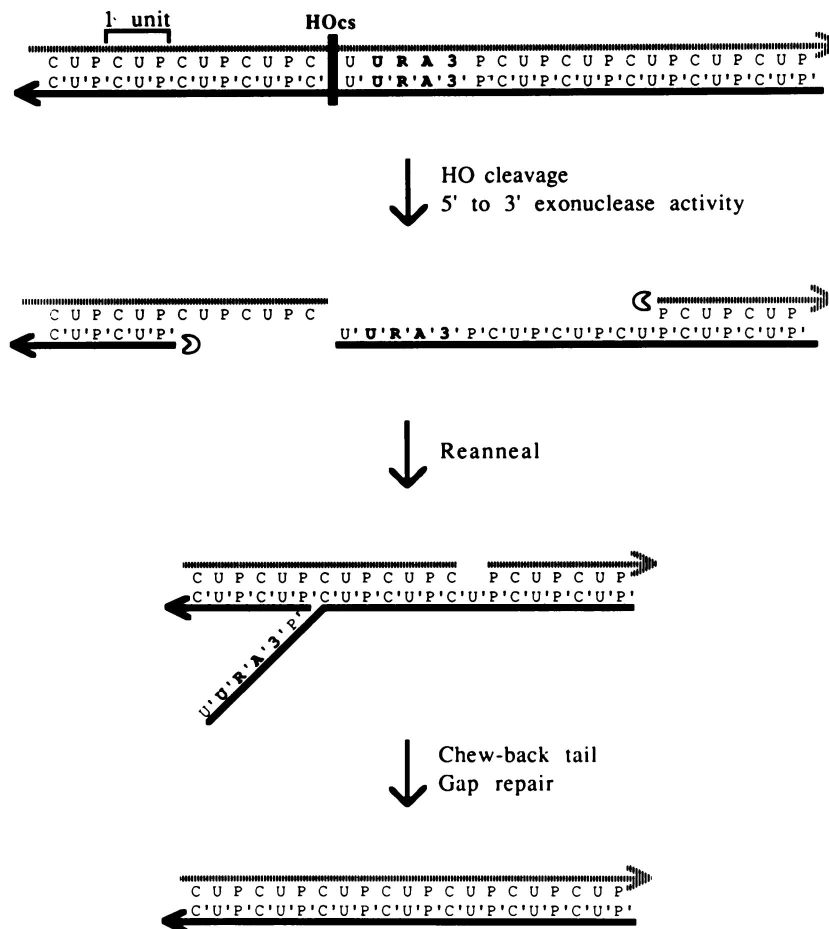


FIG. 3. Strand recision-reannealing model of DSB repair in gene arrays. An eight-unit *CUP1* gene array is shown containing the HOcs and *URA3* gene in the fourth unit from the left. Arrow heads indicate the 3′ end of each DNA strand. Complementary sequences are represented by primed and unprimed letters (e.g., CUP and C′U′P′). The details of the repair mechanism are given in the text.

fact, Rudin et al. (44) obtained evidence for preferential end invasion in their studies of DSB-induced recombination between *LACZ* repeats.

A third alternative is *RAD52*-independent DSB repair without the involvement of either end. Following the initiation of events by DNA cleavage, one repeat might recombine with any other. Such a recombination pathway could be dependent on specific factors that require a DSB to gain access to the DNA, similar to the *E. coli* RecBCD enzyme (56). The RecBCD complex is thought to enter a DNA duplex at a DSB, travel along the duplex until encountering a specific sequence (Chi site) and then initiate recombination near that site (55). DSB-induced stimulation of recombination at a site distant from the DSB has been observed in yeast cells (35). Rejoining of duplexes by this mechanism would always result in the loss of units and would usually result in the loss of *URA3*, consistent with the data. Any or all of the three mechanisms presented could account for *RAD52*-independent DSB repair, although the recision-reannealing model most favorably fits the data.

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REFERENCES

1. **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.
2. **Anderson, R. A., and S. L. Eliason.** 1986. Recombination of homologous DNA fragments transfected into mammalian cells occurs predominantly by terminal pairing. Mol. Cell. Biol. **6:**3246–3252.
3. **Bell, G. I., L. J. DeGennaro, D. H. Gelfand, R. J. Bishop, P. Valenzuela, and W. J. Rutter.** 1977. Ribosomal RNA genes of *Saccharomyces cerevisiae*. I. Physical map of the repeating unit and location of the regions coding for 5S, 5.8S, 18S, and 25S ribosomal RNAs. J. Biol. Chem. **252:**8118–8125.
4. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:**345–346.
5. **Byers, B., and L. Goetsch.** 1975. Electron microscopic observation on the meiotic karyotype of diploid and tetraploid *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **72:**5056–5060.
6. **Cao, L., E. Alani, and N. Kleckner.** 1990. A pathway for generation and processing of double-strand breaks during meiotic recombination in *S. cerevisiae*. Cell **61:**1089–1101.

7. **Chakrabarti, S., and M. M. Seidman.** 1986. Intramolecular recombination between transfected repeated sequences in mammalian cells is nonconservative. Mol. Cell. Biol. **6:**2520–2526.
8. **Christman, M. F., F. S. Dietrich, and G. R. Fink.** 1988. Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. Cell **55:**413–425.
9. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:**1582–1585.
10. **Dresser, M. E., and C. N. Giroux.** 1988. Meiotic chromosome behavior in spread preparations of yeast. J. Cell Biol. **106:**567–573.
11. **Engebrecht, J., and G. S. Roeder.** 1989. Yeast *merl* mutants display reduced levels of meiotic recombination. Genetics **121:**237–247.
12. **Fogel, S., and J. W. Welch.** 1982. Tandem gene amplification mediates copper resistance in yeast. Proc. Natl. Acad. Sci. USA **79:**5342–5346.
13. **Game, J. C., T. J. Zamb, R. J. Braun, M. A. Resnick, and R. M. Roth.** 1980. The role of radiation (*rad*) genes in meiotic recombination in yeast. Genetics **94:**51–68.
14. **Gottlieb, S., and R. E. Esposito.** 1989. A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination of ribosomal DNA. Cell **56:**771–776.
15. **Gottlieb, S., J. Wagstaff, and R. E. Esposito.** 1989. Evidence for two pathways of meiotic intrachromosomal recombination in yeast. Proc. Natl. Acad. Sci. USA **86:**7072–7076.
16. **Haber, J. E., and M. Hearn.** 1985. *RAD52*-independent mitotic gene conversion in *Saccharomyces cerevisiae* frequently results in chromosome loss. Genetics **111:**7–22.
17. **Jackson, J. A., and G. R. Fink.** 1981. Gene conversion between duplicated genetic elements in yeast. Nature (London) **292:**306–311.
18. **Jensen, R., G. F. Sprague, and I. Herskowitz.** 1983. Regulation of yeast mating type interconversion: feedback control of *HO* expression by the mating type locus. Proc. Natl. Acad. Sci. USA **80:**3035–3039.
19. **Karin, M., R. Najarian, A. Haslinger, P. Valenzuela, J. Welch, and S. Fogel.** 1984. Primary structure and transcription of an amplified genetic locus: the *CUP1* locus of yeast. Proc. Natl. Acad. Sci. USA **81:**337–341.
20. **Keil, R. L., and G. S. Roeder.** 1984. *Cis*-acting recombination-stimulating activity in a fragment of the ribosomal DNA of *S. cerevisiae*. Cell **39:**377–386.
21. **Klein, H. L.** 1988. Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae*. Genetics **120:**367–377.
22. **Lin, F.-L., K. Sperle, and N. Sternberg.** 1984. Model for homologous recombination during transfer of DNA into mouse L cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4:**1020–1034.
23. **Lin, F.-L., K. Sperle, and N. Sternberg.** 1990. Repair of double-stranded DNA breaks by homologous DNA fragments during transfer of DNA into mouse L cells. Mol. Cell. Biol. **10:**113–119.
24. **Malone, R. E.** 1983. Multiple mutant analysis of recombination in yeast. Mol. Gen. Genet. **189:**405–412.
25. **Malone, R. E., and R. E. Esposito.** 1980. The *RAD52* gene is required for homothallic interconversion of mating types and spontaneous mitotic recombination in yeast. Proc. Natl. Acad. Sci. USA **77:**503–507.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Maryon, E., and D. Carroll.** 1989. Degradation of linear DNA by a strand-specific exonuclease activity in *Xenopus laevis* oocytes. Mol. Cell. Biol. **9:**4862–4871.
28. **Nickoloff, J. A., J. D. Singer, M. F. Hoekstra, and F. Heffron.** 1989. Double-strand breaks stimulate alternative mechanisms of recombination repair. J. Mol. Biol. **207:**527–541.
29. **Orr-Weaver, T. L., A. Nicolas, and J. W. Szostak.** 1988. Gene conversion adjacent to regions of double-strand break repair. Mol. Cell. Biol. **8:**5292–5298.
30. **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.
31. **Petes, T. D.** 1979. Meiotic mapping of yeast ribosomal deoxyribonucleic acid on chromosome XII. J. Bacteriol. **138:**185–192.
32. **Petes, T. D., and D. Botstein.** 1977. Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. Proc. Natl. Acad. Sci. USA **74:**5091–5095.
33. **Prakash, L., and P. Taillon-Miller.** 1981. Effects of the *rad52* gene on sister chromatid recombination in *Saccharomyces cerevisiae*. Curr. Genet. **3:**247–250.
34. **Prakash, S., L. Prakash, W. Burke, and B. A. Montelone.** 1980. Effect of the *RAD52* gene on recombination in *Saccharomyces cerevisiae*. Genetics **94:**31–50.
35. **Ray, A., N. Machin, and F. W. Stahl.** 1989. A DNA double chain break stimulates triparental recombination in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **86:**6225–6229.
36. **Ray, A., I. Siddiqi, A. L. Kolodkin, and F. W. Stahl.** 1988. Intrachromosomal gene conversion induced by a DNA double-strand break in *Saccharomyces cerevisiae*. J. Mol. Biol. **201:**247–260.
37. **Resnick, M. A.** 1969. Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics **62:**519–531.
38. **Resnick, M. A.** 1975. The repair of double strand breaks in chromosomal DNA of yeast, p. 549–556. *In* P. C. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms for repair of DNA. Plenum Press, New York.
39. **Resnick, M. A.** 1976. The repair of double-strand breaks in DNA: a model involving recombination. J. Theor. Biol. **59:**97–106.
40. **Resnick, M. A., and P. Martin.** 1976. The repair of double-strand breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Mol. Gen. Genet. **143:**119–129.
41. **Rockmill, B., and G. S. Roeder.** 1988. *RED1*: a yeast gene required for the segregation of chromosomes during the reductional division of meiosis. Proc. Natl. Acad. Sci. USA **85:**6057–6061.
42. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101:**202–211.
43. **Rudin, N., and J. E. Haber.** 1988. Efficient repair of HO-induced chromosomal breaks in *Saccharomyces cerevisiae* by recombination between flanking homologous sequences. Mol. Cell. Biol. **8:**3918–3928.
44. **Rudin, N., E. Sugarman, and J. E. Haber.** 1989. Genetic and physical analysis of double-strand break repair and recombination in *Saccharomyces cerevisiae*. Genetics **122:**519–534.
45. **Schild, D., I. L. Calderon, R. Contopoulou, and R. K. Mortimer.** 1983. Cloning of yeast recombination repair genes and evidence that several are nonessential genes, p. 417–427. In E. C. Friedberg and B. A. Bridges (ed.), Cellular responses to DNA damage. Alan R. Liss, New York.
46. **Schild, D., B. Konforti, C. Perez, W. Gish, and R. Mortimer.** 1983. Isolation and characterization of yeast DNA repair genes. I. Cloning of the *RAD52* gene. Curr. Genet. **7:**85–92.
47. **Schweizer, E., C. Mackechnie, and H. O. Halvorson.** 1969. The redundancy of ribosomal and transfer RNA genes in *Saccharomyces cerevisiae*. J. Mol. Biol. **40:**261–277.
48. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics: laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
49. **Sokal, R. R., and R. J. Rohlf.** 1981. Biometry. W. H. Freeman and Co., New York.
50. **Stillman, D. J., A. T. Bankier, A. Seddon, E. G. Groenhout, and K. A. Nasmyth.** 1988. Characterization of a transcription factor involved in mother cell specific transcription of the yeast *HO* gene. EMBO J. **7:**485–494.
51. **Strathern, J. N., A. J. S. Klar, J. B. Hicks, J. A. Abraham, J. M. Ivy, K. A. Nasmyth, and C. McGill.** 1982. Homothallic switching of yeast mating type cassettes is initiated by a double-strand cut in the *MAT* locus. Cell **31:**183–192.
52. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1979. High-frequency transformation of yeast: autonomous replica-

tion of hybrid molecules. Proc. Natl. Acad. Sci. USA **76**:1035–1039.

53. **Sun, H., D. Treco, N. P. Schultes, and J. W. Szostak.** 1989. Double-strand breaks at an initiation site for meiotic gene conversion. Nature (London) **338**:87–90.
54. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** 1983. The double-strand-break repair model for recombination. Cell **33**:25–35.
55. **Taylor, A. F., D. W. Schultz, A. S. Ponticelli, and G. R. Smith.** 1985. RecBC enzyme nicking at Chi sites during DNA unwinding: location and orientation-dependence of the cutting. Cell **41**:153–163.
56. **Thaler, D. S., and F. W. Stahl.** 1988. DNA double-chain breaks in recombination of phage λ and of yeast. Annu. Rev. Genet. **22**:169–197.
57. **Weiffenbach, B., and J. E. Haber.** 1981. Homothallic mating type switching generates lethal chromosome breaks in *rad52* strains of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1**:522–534.
58. **Weiffenbach, B., D. T. Rogers, J. E. Haber, M. Zoller, D. W. Russell, and M. Smith.** 1983. Deletions and single base pair changes in the yeast mating type locus that prevent homothallic mating type conversions. Proc. Natl. Acad. Sci. USA **80**:3401–3405.
59. **White, C. I., and J. E. Haber.** 1990. Intermediates of recombination during mating type switching in *Saccharomyces cerevisiae*. EMBO J. **9**:663–673.
60. **Zamb, T. J., and T. D. Petes.** 1981. Unequal sister-strand recombination within yeast ribosomal DNA does not require the *RAD52* gene product. Curr. Genet. **3**:125–132.