Efficient Repair of HO-Induced Chromosomal Breaks in *Saccharomyces cerevisiae* by Recombination between Flanking Homologous Sequences

NORAH RUDIN† AND JAMES E. HABER*

Department of Biology and Rosenstiel Basic Medical Sciences Research Center, Brandeis University,
Waltham, Massachusetts 02254

Received 18 April 1988/Accepted 17 June 1988

Novel recombinational repair of a site-specific double-strand break (DSB) in a yeast chromosome was investigated. When the recognition site for the HO endonuclease enzyme is embedded in nonyeast sequences and placed between two regions of homology, expression of HO endonuclease stimulates recombination between the homologous flanking regions to yield a deletion, the apparent product of an intrachromosomal exchange between direct repeats. This deletion-repair event is very efficient, thus preventing essentially all the potential lethality due to the persistence of a DSB. Interestingly, unlike previous studies involving spontaneous recombination between chromosomal repeats, the recombination events stimulated by HO-induced DSBs are accompanied by loss of the sequences separating the homologous regions greater than 99.5% of the time. Repair is dependent on the *RAD52* gene. The deletion-repair event provides an in vivo assay for the sensitivity of any particular recognition site to HO cleavage. By taking advantage of a galactose-inducible *HO* gene, it has been possible to follow the kinetics of this event at the DNA level and to search for intermediates in this reaction. Deletion-repair requires approximately 45 min and is inhibited when cycloheximide is added after HO endonuclease cleavage.

The repair of double-strand breaks (DSBs) in *Saccharomyces cerevisiae* seems to occur predominantly by recombination between the broken ends of a DNA molecule and intact, homologous sequences. When chromosomal DSBs are induced by gamma-irradiation, efficient repair depends on the presence of either an unbroken homologous chromosome in a diploid or an intact sister chromatid in a haploid cell after DNA replication (36, 37). Similarly, the repair of spontaneous breaks at or near the *MAT* locus in heterothallic (*ho*) strains frequently occurs by recombination between the fragment of broken chromosome containing the centromere and the intact homologous chromosome (27); in some cases, complete loss of the broken chromosome is observed. Additionally, the repair of dicentric chromosomes, broken during mitosis, was also often observed to occur by recombination between one end of the DSB and a homologous segment elsewhere on the remaining monocentric broken chromosome (11). Orr-Weaver et al. (33) showed that a plasmid that had been linearized in vitro was highly recombinogenic when transformed into yeast cells; several different types of interaction with a homologous region of the genome, which lead to repair, were observed, depending on whether the initial molecule contained a gap or a simple cut (32). In a fashion analogous to the interchromosomal interactions discussed above, a linear plasmid, restriction-cut within a telomeric Y' element, may heal itself by recombination between one end of the cleaved Y' region and an intact Y' region at the end of a chromosome (5).

Homothallic mating-type switching represents a very specialized site-specific chromosomal DSB repair event. The HO endonuclease (21, 22) makes a DSB at a particular

sequence at the *MAT* locus (18, 46, 50), which in normal haploid strains is repaired by an intrachromosomal gene conversion event between *MAT* and one of the two donors, *HML* α and *HMR* α (9, 19, 29). In a haploid homothallic strain in which both *HML* and *HMR* have been deleted, an HO-induced DSB at *MAT* has been shown to be lethal, presumably because no homologous sequences are available from which to repair the break (18). In diploids lacking the donor loci, and in which only one of the two *MAT* genes can be cleaved by HO endonuclease, the broken chromosome is repaired by gene conversion from the intact *MAT* locus. Unlike a normal mating-type switch, repair associated with crossing-over and/or homozygosis of the distal arm of chromosome III occurred in over 25% of the cases (17).

Although most DSB repair in yeast cells proceeds by homologous recombination, a low frequency of apparently nonhomologous repair events has been described. Small deletions surrounding the HO cut site sometimes occur as a consequence of attempted mating type switching in *rad52* or donor-deleted cells (18, 50). When linearized plasmids lacking homology to the yeast genome are used for transformation, most repair events appear to be promoted by the intermolecular pairing of two linear duplexes, resulting in formation of head-to-head dimers of the original plasmid, rather than simple recircularization (23).

A last possible mechanism of healing broken chromosomes involves the formation of a new telomere. In several studies, terminally deficient chromosomes that may have become stable by the de novo addition of a yeast telomere have been observed (5, 11, 27); the discovery of a template-independent terminal transferase in *Tetrahymena* spp. (7) supports this possibility. Other mechanisms of telomere addition might involve recombination between dispersed, homologous sequences.

Both spontaneous and induced homologous DSB repair depend on several genes identified by their radiation sensi-

* Corresponding author.

† Present address: Cell and Molecular Biology Division, Lawrence Berkeley Laboratory 934-47A, University of California, Berkeley, Berkeley, CA 94720.

tivity (6, 28, 35). Specifically, the *RAD52* gene product has been defined as a necessary element in all homologous repair events that have been studied (5, 10, 15, 25, 32, 33, 37, 40, 50). In *rad52* diploids, in which only one *MAT* locus can be cut by HO endonuclease, the broken chromosome is almost always lost. HO-induced breaks in *rad52* haploids can be rescued by mating the cells to a *Rad*⁺ strain. In the latter situation, repair of the broken chromosome generates a spectrum of events similar to those of the donorless strains described above (17, 50; K. Kramer, M. Klucznik, and J. E. Haber, unpublished data).

To date, the only method of inducing a site-specific DSB in yeast cells, *in vivo*, is by using the HO endonuclease recognition site. A small portion of the *MAT* locus, containing the HO endonuclease recognition site, has previously been shown to induce recombination events when HO is expressed. HO-stimulated recombination has been observed both mitotically (31) and meiotically (20) when the cut site is inserted in a heterologous region of DNA. In those studies, the HO cut site was always situated within one of the regions involved in the homologous interaction. We have examined HO-induced events in constructions in which a *MAT* α cut site was integrated on chromosome V between two homologous segments. In greater than 99.5% of the cases observed, HO induction of a DSB in this situation resulted in recombination between the surrounding regions of homology accompanied by deletion of the sequences between them. This highly efficient deletion-repair event provides an *in vivo* assay for the sensitivity of any particular recognition site to HO cleavage; the effects of specific mutations made within a defined cut site may be easily measured in this fashion. By taking advantage of a galactose-inducible HO gene, the kinetics of this event at the DNA level were monitored to reveal intermediates in the reaction and to assess the effects of an inhibitor of the repair event.

MATERIALS AND METHODS

Plasmids. Plasmid pJH124 was constructed by inserting the 2.2-kilobase (kb) *XhoI-SalI* *LEU2* fragment into a *XhoI* linker deletion of *MAT* α . The *XhoI* linker strains used to construct the deletion, α 161 and α 124, were a gift from K. Tatchell (48). The *LEU2* gene replaces sequences from the middle of the W homology block through the Z1 region. Plasmid pJH2 consists of the 1.2-kb *HindIII* *URA3* fragment inserted into the *HindIII* site of pBR322 and an *EcoRI-HindIII* *MAT* α fragment, in direct orientation relative to *URA3* inserted between the *EcoRI* and *HindIII* sites. Plasmid pRK113 (a gift from R. Kostriken) consists of a 117-base-pair (bp) *HincII-BglIII* fragment containing the *MAT* α HO cut site inserted into *BamHI-HincII*-cut pUC9 (22). Plasmids pNR6 and pNR7 were constructed by inserting, in either orientation, a *HindIII* *URA3* fragment into the *HindIII* site of the multiple cloning site of plasmid pRK113. The *EcoRI* *ARS* fragment was removed from plasmid pLG199 *Leu* (2 μ m *URA3* *CYC1* *UAS/LACZ*), a gift from L. Guarente (8), and the *Tth1III* site was changed to a *HindIII* site by using linkers. The *HincII* junction of plasmid pRK113 was changed to a *BamHI* site by using linkers, and the fragment was released by using *XhoII*, which will cut at a *BamHI* site as well as a *BamHI-BglIII* junction. Plasmid pNR14 was obtained by inserting the *XhoII* *MAT* α cut-site fragment into the *BclI* site of *LACZ*. It was subsequently discovered that two copies of the cut site, in direct orientation, had been inserted (*LACZ::CS*₂). Both 117-bp *BglIII-HincII* *MAT* α cut sites are oriented with the *MATZ1* region (originally *HincII*)

closest to the 5' side of *LACZ*. Plasmid pJH211 consists of a 20-bp synthetic oligomer, including the *MAT* α cut site (31), inserted in the *BclI* site of the *LACZ* gene in plasmid pJH127.

Plasmid pSE271::*GAL10::HO* contains a *GAL10::HO* fusion on a *TRP1* *ARS1* *CEN4* vector (31) and was a gift from the laboratory of F. Heffron. It is referred to as pJH283.

Plasmid pSM20 is a *rad52::LEU2* disruption (gift of D. Schild) (42).

Plasmid YIp30 (1) contains the 1.2-kb *HindIII* *URA3* fragment inserted into the *HindIII* site of pBR322. Plasmid YIp30 and gel-purified *HindIII* *URA3* fragment were labeled with [γ -³²P]dCTP by nick translation and used as probes for Southern blot hybridization.

Strains. Strains used in this study are listed in Table 1. Strain DBY745 was originally obtained from D. Botstein. Strain tNR35 (Fig. 1) was constructed by replacing (39) the *MAT* α allele of DBY745 with a *mat* Δ ::*LEU2* deletion from plasmid pJH124. Strains tNR36 (Fig. 1), tNR64, and tNR65 (see Fig. 3) are integrations into strain tNR35 at *ura3-52* of plasmids pJH2, pNR6, and pNR7, respectively. Strains tNR60 and tNR67 (see Fig. 4) are integrations of plasmids pJH211 and pNR14, respectively, at *ura3-52* in strain DBY745. Strain tNR85 was constructed in our laboratory by replacing the normal *MAT* α allele of strain R126 (*ho* *HML* α *MAT* α *hmr-3* Δ *leu2-3,112* *ura3-52* *trp1* *thr4* *GAL*) with *mat* Δ ::*LEU2*. This strain was subsequently transformed with plasmid pJH283. The *hmr-3* Δ was originally obtained from A. J. S. Klar (16). Strain tNR111 was obtained by integrating plasmid pNR6 at *ura3-52* in strain tNR85. Strain NR151-25D is a homothallic (*HO*) diploid recovered from cross NR151. A *Leu*⁻ version (NR151-25D*Leu*⁻) was obtained from an ectopic meiotic gene conversion which arose in a dissection of strain NR151-25D. The *rad52::LEU2* disruption was introduced by transformation with a *BamHI* fragment from plasmid pSM20. All other strains were constructed in this laboratory.

Media. Cells were grown in YEP (1% [wt/vol] yeast extract, 2% [wt/vol] Bacto-Peptone) supplemented with dextrose (2% [wt/vol]) (YEPD) or lactic acid (3.7%, pH 5.5), (YEPL). When required, galactose (2%, wt/vol) was added as a 20% solution (wt/vol) to YEPL medium. Synthetic complete medium lacking uracil, leucine, or tryptophan and minimal medium plus threonine was made as described by Sherman et al. (43). Bacto-Agar was added (2%) for solid media.

Yeast transformation. Yeast cells were transformed with lithium acetate by the method of Ito et al. (14).

HO induction. A stationary-phase culture grown in media selective for the plasmid(s) was diluted 1:100 into YEP-lactate to yield a final concentration of approximately 5×10^5 cells per ml. The cultures were grown at 30°C with vigorous aeration to a final cell density of 1×10^7 to 3×10^7 cells per ml. Galactose was added as a 20% (wt/vol) solution, and the incubation was continued. Cells were removed at various times for subsequent analysis.

DNA extraction. DNA was extracted in the following manner. Cultures (25 ml; 5×10^7 to 10×10^7 cells) were collected on an 0.45- μ m-pore-size nitrocellulose filter by filtration and washed with 1 to 2 ml of 10 mM Tris hydrochloride-1 mM EDTA, pH 7.4 (TE). Cells were washed from the filter with 0.5 ml of 100 mM Tris hydrochloride (pH 8.0)-50 mM EDTA-2% sodium dodecyl sulfate into a 1.5-ml microcentrifuge tube. Glass beads (0.45- to 0.5-mm diameter, acid washed and baked) were added to the level of the meniscus. TE-saturated phenol (0.5 ml) was added, and the tube was vortex mixed twice for 15 s and then cooled on

was electrophoresed on 0.5% agarose gels at 40 to 60 V for about 17 h, transferred to nitrocellulose filters (Millipore Corp.), hybridized to [γ - ^{32} P]dCTP-labeled probe, washed, and autoradiographed by standard procedures (26, 44). Nick-translated probes were prepared by standard procedures.

Scoring of *HO*. When necessary, *HO* was scored by taking advantage of the strains U60 and U90 as described by Weiffenbach and Haber (50).

RESULTS

An HO-induced break situated between repeated sequences efficiently stimulates homologous recombination. A strain was constructed which permitted the study of HO-initiated DSBs which were not resolved by mating-type switching. The three mating-type cassettes are normally all found on chromosome III (9, 19, 29). By using gene replacement, the normal copy of *MAT* on chromosome III in strain DBY745 (*ho MATa leu2-3,112 ura3-52 ade1-100*) was almost entirely replaced by a 2.2-kb fragment of *LEU2* (*matΔ::LEU2*) (Fig. 1A). This construction renders the cell *Mata*⁻, since no functional mating-type information remains, as well as unable to switch, since the HO endonuclease recognition site has been removed (45, 51). A pBR322 plasmid containing *MATa* and *URA3* (pJH2) was integrated at the *ura3-52* locus on chromosome V, yielding a duplication of the *URA3* genes surrounding the *MAT/pBR322* sequences (tNR36) (Fig. 1B). This construction provided a situation in which an HO-induced DSB might be repaired via an interchromosomal switch by using the donors on chromosome III (Fig. 1C) or by an alternative homologous or nonhomologous interaction; an unrepaired chromosomal DSB would probably be lethal (18). A homothallic (*HO*) derivative of tNR36 was obtained by crossing it to the *HO MATa-inc* (34) strain, U189, and dissecting the diploid, NR151. Homothallic diploids of the genotype *HO/HO matΔ::LEU2/matΔ::LEU2 ura3-52-MATa-URA3/ura3-52-MATa-URA3* were identified as nonmating (NM) *Leu*⁺ *Ura*⁺ colonies and confirmed by Southern analysis (data not shown). *HO* is repressed in *MATa/MATa* diploids and becomes active only during germination of the meiotic haploid products. One segregant, NR151-25D, was selected from this cross, and its meiotic progeny were analyzed for the outcome of an HO-induced DSB on chromosome V. This diploid was first analyzed by tetrad analysis. If the DSB was repaired by switching from the donors on chromosome III, the resultant *ura3-52-MATa-URA3* daughter cells (Fig. 1C) would mate with their unswitched *ura3-52-MATa-URA3* mothers, producing four diploid segregants with stable *Leu*⁺, *Ura*⁺, NM phenotypes; any deviation in the mode of repair might be detected by colonies that showed something other than an NM phenotype. Many of the colonies were *Ura*⁻ or weakly *Ura*⁺ as well as showing an *a*-mating phenotype (Table 2). Examination of subclones of the *a*-mating *Ura*⁺ colonies revealed a mixture of phenotypes, consisting of NM *Ura*⁺ cells and *a*-mating *Ura*⁻ cells (Table 2). In addition, the *a*-mating colonies were found to be *Mata*⁻, indicating that they in fact contained no functional mating-type information. As expected, the NM *Ura*⁺ strains were found to be stable *ura3-52-MATa-URA3/ura3-52-MATa-URA3* diploids.

Several *Mata*⁻ *Ura*⁻ and NM *Ura*⁺ subclones were examined by Southern analysis (Fig. 2). Genomic DNA was digested with *Bam*HI, which lacks a recognition sequence in either *URA3* or *Ty*, and probed with a *URA3/pBR322* plasmid (YIp30). The *ura3-52* allele produced a 11.5-kb fragment, while the duplication was visualized as an 11-kb

TABLE 2. Loss of *Ura*⁺ in segregants from strain NR151-25D^a

Organism type	No. of organisms with phenotype ^b :		
	NM <i>Ura</i> ⁺	<i>Mata</i> <i>Ura</i> ⁻	<i>Mata</i> <i>Ura</i> ⁺
Segregants	32	102	50
Subclones ^c	25	15	0
	0	40	0
	4	36	0
	6	34	0
	22	18	0
	8	32	0
	2	38	0
	5	32	3
	0	36	4
	5	35	0

^a NR151-25D *HO matΔ::LEU2 ura3-52-MATa-URA3*

HO matΔ::LEU2 ura3-52-MATa-URA3. Data were compiled from results from 46 tetrads comprising a total of 184 segregants. Dissection of NR151-25D generally resulted in 75 to 80% of tetrads with four viable spores.

^b *Mata*, *a*-Mating. *Mata* subclones were found to lack *MATa* information as a result of plasmid loss and thus were in fact *Mata*⁻; NM phenotype results from successful switching of *ura3-52-MATa-URA3* to *ura3-52-MATa-URA3* and subsequent mating to unswitched mother cells.

^c 40 subclones from each of 10 *Mata* *Ura*⁺ colonies were examined. The percentages of total NM *Ura*⁺, *Mata* *Ura*⁻, and *Mata* *Ura*⁺ subclones were 19, 79, and 2%, respectively.

band plus a 9.2-kb band because of a *Bam*HI site in pBR322 sequences. The *Mata*⁻ *Ura*⁻ subclones clearly showed the parental *ura3-52* band, while the NM *Ura*⁺ subclone gave the bands expected for the integrant. These results are consistent with loss of the integrated plasmid sequences via recombination between the duplicated *URA3* sequences. Homologous recombination apparently has a much higher chance of occurring 3' to the *Ty* element and resulting in loss of the internal sequences. This may be partly due to the fact that the position of the *Ty* element in *ura3-52* leaves 70% more homology on the 3' side (38); it is also possible that the 6 kb of heterology introduced by the *Ty* element biases

FIG. 2. Southern analysis of *Mata*⁻ *Ura*⁻ subclones. DNA was isolated from four *Mata*⁻ *Ura*⁻ subclones and one NM *Ura*⁺ subclone derived from NR151 as well as the parent strain, DBY745. The DNA was digested with *Bam*HI (see the legend to Fig. 1), and the fragments were separated on an agarose gel and transferred to nitrocellulose membrane. The filter was hybridized with a *URA3/pBR322* plasmid (YIp30), which was labeled with [γ - ^{32} P]dCTP by nick translation. A 11.5-kb band corresponding to the *ura3-52* gene is observed in lanes 2 through 5, which contain DNA from *Mata*⁻ *Ura*⁻ subclones, as well as in lane 1, which contains DNA from DBY745. Lane 6 contains DNA from a NM *Ura*⁺ subclone, in which the 9.2- and 11-kb bands which are indicative of the intact duplication are evident.

FIG. 3. Results of an HO-induced break in strains tNR64 and tNR65. Plasmid pNR6 or pNR7 was integrated into DB745 at *ura3-52* to produce, respectively, tNR64 (B) or tNR65 (A). Integrations were directed by digestion with *Sst*I. The two possible outcomes of an HO-induced cut are (i) restoration of the *ura3-52* gene (C) and (ii) production of the *URA3* gene (very infrequent) (D). The short vertical arrows represent *Bam*HI sites.

resolution of the event to the side proximal to the *URA3* gene. In either case, the end result of this interaction left a single *ura3-52* gene on the chromosome (Fig. 1D). Although the vast majority (97%) of plasmid loss events resulted in a *Mata⁻ Ura⁻* phenotype, a small number (3%) gave a *Mata⁻ Ura⁺* phenotype. In a separate experiment discussed below, these products were in fact found to result from a crossover 5' to the Ty element, restoring an intact *URA3* gene. We envision this novel event as a result of an HO endonuclease-cut *MAT* locus, which fails to pair with either of the donors located on chromosome III. A potentially lethal DSB is instead rescued by homologous recombination between the surrounding *URA3* sequences.

An HO-initiated homologous recombination event is very efficient. Although it was clear that most of the segregants of NR151-25D gave rise to CFU, it was still possible that in a significant fraction of the cells in the population, the HO-induced DSB was not repaired and resulted in lethality as described by Klar et al. (18). Pedigree analysis of individual cells was performed to establish the efficiency of the recombinational rescue. Tetrads of NR151-25D were dissected in the presence of the mating pheromone, α -factor. Germinating *MAT α* spores, which continue to grow, are easily distinguished from *MAT α* spores, which arrest with a distinctive shmoo phenotype before their first division. Twelve *MAT α* spores were observed microscopically through two cell divisions. In 10 of 12 cases, the mother cell and its second daughter both became α -factor sensitive, while the first daughter and its daughter cell remained able to bud. This is the expected pattern of homothallic switching (13). The 10 pairs of switched cells were then removed from the vicinity of the α -factor and allowed to grow. All 20 cells grew to form viable colonies, 16 of which were *Mata⁻ Ura⁻*. Of the other four colonies, two showed a NM *Ura⁺* phenotype, while two were mixed *a*-mating *Ura⁺* colonies. Thus, in the 16 cases where *MAT α* failed to switch to *MAT α* , there was no observable "pedigree of death" despite the fact that they suffered a DSB. The original daughter cells, still *MAT α* , were also observed microscopically for an additional generation. They continued to divide with no observable cell death. We conclude from this that the deletion-repair event is quite efficient.

The deletion-repair event is dependent on the *RAD52* gene product. It has previously been shown that homothallic cells die in the presence of the *rad52* mutation, while heterothallic cells are not affected (25, 50). To test whether the HO-induced deletion-repair event was also *RAD52* dependent, a *rad52* mutation was introduced into a strain derived from, and isogenic to NR151-25D. A homothallic diploid segregant from the dissection of NR151-25D (NR151-25DLeu⁻), which had become Leu⁻ at *mat Δ ::LEU2* due to an ectopic meiotic gene conversion from the *leu2* gene (24), was transformed with a *rad52::LEU2* gene disruption. Since the transformation was performed in a diploid, a strain was obtained in which only one homolog contained the *rad52::LEU2* gene, while the other remained wild type (data not shown), providing an internal control. When such a diploid (tNR116) was dissected, 14 tetrads all produced only two viable spores which were both Leu⁻, indicating that *rad52::LEU2* spores were inviable. These results established that the HO-induced deletion-repair event was dependent on the *RAD52* gene product, as expected for DSB repair.

A 117-bp fragment containing the *MAT α* cut site stimulates recombinational repair. The deletion-repair event provides a sensitive in vivo assay for the efficiency with which an HO endonuclease site is cut, since any susceptible site located between the *URA3* sequences will very efficiently produce *Ura⁻* cells in the presence of the endonuclease. A plasmid containing a 117-bp *Bgl*III-*Hinc*II *MAT α* fragment (pRK113), encompassing the cut site, has been shown to be cut in vivo by HO endonuclease (21, 22). A 1.2-kb *URA3* fragment was inserted into this plasmid (pNR6) so that it could be integrated into DB745 at *ura3-52* (tNR64) (Fig. 3B) in an analogous fashion to the *MAT/URA3* plasmid. Through a series of crosses, an *HO/HO MAT α -inc/MAT α* diploid (NR 232) heterozygous for the HO cut site integration was obtained and analyzed (Table 3). When only the *MAT α -inc* segregants were considered (The *MAT α -inc* allele is not cut by HO, so that the only HO-sensitive site in each cell is on chromosome V), each was entirely *Ura⁻*, indicating that cutting of the HO recognition site, as well as the repair event, was taking place efficiently. The same set of experiments were performed with the HO cut site in the reverse orientation relative to the *URA3* sequences (pNR7, tNR65)

TABLE 3. Stimulation of recombination by the 117-bp *MATa* cut site

Initial genotype of segregant	Expected no. of segregants from:		Phenotype ^a	No. of segregants from:	
	NR232 ^b	NR233 ^c		NR232	NR233
<i>MATa ura3</i>	15.5	16	NM Ura ⁻	13	17
<i>MATa ura3-52-cut site-URA3</i>	15.5	16	NM Ura ⁺	18	15
<i>MATa-inc ura3</i>	15.5	16	<i>Matα Ura</i> ⁻ } <i>Matα Ura</i> ⁻ }	31 ^d	32 ^d
<i>MATa-inc ura3-52-cut site-URA3</i>	15.5	16			

^a *Matα*, α-Mating.^b NR232 *HO MATa ura3-52-cut site-URA3*. Data were compiled from results from 11 tetrads with four viable spores and 6 tetrads with three viable spores, comprising a total of 62 segregants.^c NR233 *HO MATa ura3-52-cut site-URA3*. Data were compiled from results from 10 tetrads and 8 triads comprising a total of 64 segregants.^d Total for *Matα-inc ura3* and *Matα-inc ura3-52-cut site-URA3*.

(Fig. 3A). The new diploid, NR323, was analyzed as described above (Table 3), and exactly the same results were obtained as with NR322, suggesting that the outcome of the deletion-repair event is independent of orientation of the HO cut site relative to the *URA3* sequences involved in the interaction.

A 20-bp synthetic HO recognition site is not cut in vivo. Using the assay described above, we have shown that a 20-bp sequence (31), including a *MATa* cut site, shows no detectable cutting in vivo. The oligonucleotide sequence in the plasmid, CAGCTTTCGCAACAGTAAA, includes 8 bp of the *Yα* region and 12 bp of *Z1*. In vitro, this sequence is cleaved less efficiently than an intact *MATα* sequence by HO endonuclease purified from *Escherichia coli* (31). Both the 20-bp oligomer (pJH211) and the 117-bp fragment (pNR14) were inserted into the *Bcl*I site of a *LACZ* gene, on a plasmid that also contained the *URA3* gene (see Materials and Methods). In pNR14, the *LACZ* gene contains a tandem repeat (117₂) of the HO cut site. These two plasmids were each integrated at *ura3-52* in strain DBY745 (tNR60, tNR67) (Fig. 4). By crosses similar to those described above, *HO MATa-inc ura3-52-LACZ::CS(20)-URA3* (diploid NR219) and *HO MATa-inc ura3-52-LACZ::CS(117₂)-URA3* (diploid NR245) derivatives were obtained. A total of 14 *HO MATa-inc* segregants from NR245 were analyzed (Table 4), one half of which should have initially contained the *URA3* duplication. All 14 segregants were completely Ura⁻, indicating that the efficiency with which the 117-bp cut site was cleaved was not appreciably affected by embedding the site in *LACZ* sequences. Of the 31 *MATa-inc* segregants from NR219 (Table 4B), all showed a Ura⁺ phenotype, indicating a reduction in HO cleavage, and about one half (16) should have contained the *HO* gene. A total of 18 were tested for

HO (see Materials and Methods), and 9 were found to be positive, confirming this supposition. Thus, the 20-bp cut site is apparently cut at a reduced frequency compared with the 117-bp cut site.

A more quantitative measure of the inefficient cutting of the 20-bp recognition site was obtained by subcloning Ura⁺ *HO MATa-inc ura3-52-(CS)20-URA3* segregants and determining the frequency of Ura⁻ colonies in the population. Six independent *HO* segregants were subcloned, and several independent Ura⁺ colonies from each were grown on YEPD. Each Ura⁺ colony was then subcloned on YEPD and replica plated to Ura⁻ plates. Among 24 independent colonies examined, the frequency of Ura⁻ subclones averaged 0.009. This value is 10-fold higher than that obtained when an equivalent number of *ho MATa-inc ura3-52-(CS)20-URA3* segregants were analyzed in a similar fashion (0.0008). Thus, the 20-bp cut site is recognized and cleaved in vivo, but at approximately 1% of the frequency of the 117-bp cut site. This difference is markedly greater than the relative efficiency with which this same 20-bp segment is cleaved by HO endonuclease in vitro (31).

The *URA3* deletion event shows similar characteristics when initiated by galactose-inducible HO endonuclease. pNR6 was also integrated at *ura3-52* into a heterothallic strain (tNR85) which carries a plasmid-borne galactose-inducible *HO* gene, pJH283 (see Materials and Methods). This strain (tNR111) is deleted for *MAT* and *HMR*; since the cloned cut site is from the *MATa* allele, interaction with *HMLα* should be negligible, because the only homology remaining is 67 bp of the *Z* region to one side of the cut. The *pGAL10::HO* system allows synchronous induction of HO endonuclease in a large population of cells, as well as providing for propagation of the strain in a stable uninduced state. Galactose inductions of HO endonuclease were performed as described in Materials and Methods. When tNR111 was allowed to grow overnight in galactose, 926/1001 (97.5%) of the Trp⁺ CFU were Ura⁻, indicating that the deletion-repair event also took place very efficiently when *HO* was expressed from the *GAL10* promoter. The number of CFU per plate was approximately as predicted from a previous hemacytometer count, further confirming the efficiency of this event (data not shown). A total of 25/1001 (2.5%) CFU were both Ura⁺ and Trp⁺, indicating that they retained both the *GAL10::HO* plasmid and the *ura3-52-cut site-URA3* duplication. Of these, 16 (1.5%) were unstable for Ura⁺ when reexposed to galactose, indicating that they had not been cut during the first induction, and 9 (1.0%) were stable Ura⁺ strains. Six of these were examined by Southern analysis, and five were

FIG. 4. Structures of strains tNR60 and tNR67. Plasmids pJH211 (20-bp cut site synthetic oligomer) and pNR14 (117-bp *MATa* cut site) were integrated into DBY745 at *ura3-52* to produce, respectively, tNR60 and tNR67. Integrations were directed by digestion with *Stu*I.

TABLE 4. Comparison of stimulation of recombination by the 117-bp *MATa* cut site or a 20-bp synthetic oligomer in *LACZ*

Strain	Initial genotype of segregant	Expected no. of segregants	Phenotype ^a	No. of segregants
NR245 ^b	<i>ho MATα ura3-52-LACZ::CS-URA3</i>	7	<i>Mata Ura⁺</i>	8
	<i>ho MATα ura3</i>	7	<i>Mata Ura⁻</i>	7
	<i>ho MATa-inc ura3-52-LACZ::CS-URA3</i>	7	<i>Mata Ura⁺</i>	6
	<i>HO MATα ura3-52-LACZ::CS-URA3</i>	7	<i>NM Ura⁺</i>	8
	<i>HO MATα ura3</i>	7	<i>NM Ura⁻</i>	7
	<i>HO MATa-inc ura3</i>	7	<i>Mata Ura⁻</i>	21
	<i>ho MATa-inc ura3</i>	7	<i>Mata Ura⁻</i>	
	<i>HO MATa-inc ura3-52-LACZ::CS-URA3</i>	7	<i>Mata Ura⁻</i>	
NR219 ^c	<i>ho MATα ura3-52-LACZ::CS-URA3</i>	15.5	<i>Mata Ura⁺</i>	16
	<i>ho MATα ura3</i>	15.5	<i>Mata Ura⁻</i>	21
	<i>HO MATα ura3-52-LACZ::CS-URA3</i>	15.5	<i>NM Ura⁺</i>	12
	<i>HO MATα ura3</i>	15.5	<i>NM Ura⁻</i>	13
	<i>HO MATa-inc ura3</i>	15.5	<i>Mata Ura⁻</i>	31
	<i>ho MATa-inc ura3</i>	15.5	<i>Mata Ura⁻</i>	
	<i>ho MATa-inc ura3-52-LACZ::CS-URA3</i>	15.5	<i>Mata Ura⁺</i>	31 ^d
	<i>HO MATa-inc ura3-52-LACZ::CS-URA3</i>	15.5	<i>Mata Ura⁺</i>	

^a *Mata*, α-Mating; *Mata*, a-mating. *HO MATα* segregants almost always give rise to a nonmating (NM) *Ura⁺* phenotype for the following reasons. (i) A switch and subsequent conjugation to form a *MATa/MATα* diploid might shut down *HO* synthesis before the cut site integrated on chromosome V is acted upon. (ii) Most diploids should result from mating between a mother cell in which *HO* has been expressed and a daughter cell in which it has not (30).

^b NR245 *ho leu2 MATα* + *adel ura3-52-LACZ-CS(117)-URA3*
HO leu2 MATa-inc lys5 adel ura3

. Data were compiled from results from 14 tetrads, comprising a total of 56 segregants.

^c NR219 *ho leu2 MATα* + *adel* + *ura3-52-LACZ::CS(20)-URA3*
HO leu2 MATa-inc + lys5 ura3

. Data were compiled from results from 19 tetrads and 16 triads, comprising a total of 124 segregants.

^d Of the 31 *Mata*, *Ura⁺* segregants, 18 were tested for *HO* by mating to U60, as described in Materials and Methods, and 9 were confirmed as positive.

found to have retained only the *URA3* allele on the chromosome, presumably by a crossover event 5' to the *Ty* element. One produced a different rearrangement which was not investigated further (data not shown). Thus, an homologous recombination event producing the *Ura⁺* allele on the chromosome occurred only about 1% of the time and in fact was very difficult to detect by traditional genetic analysis.

Kinetic analysis of an *HO*-induced homologous recombination event. By taking advantage of *pGAL10::HO*, it was now possible to follow the kinetics of various recombination events over a period of time. tNR111 was first examined genetically by taking samples from a galactose-induced culture over a 6-h period and plating onto YEPD plates. Colonies were then replica-plated to medium lacking tryptophan to determine which cells still retained the *pGAL10::HO* plasmid and medium lacking uracil to determine when commitment to the deletion-repair event had occurred. Table 5 shows a 6-h genetic time course for tNR111. By 1 h, about two-thirds of the population was apparently committed to completion of the deletion-repair event. Loss of the *Ura⁺*

phenotype continued to increase through 6 h, at which point almost all the *Trp⁺* cells had lost the *URA3* duplication.

The physical intermediates of this induction are readily examined by extracting DNA at various time points and performing Southern analysis. Figure 5 shows such a time

TABLE 5. Genetic time course of a deletion-repair event^a

Time (h)	CFU	No. of <i>Trp⁺</i> ^b	No. of <i>Trp⁺</i> <i>Ura⁻</i> /total <i>Trp⁺</i> (%)
0	424	405	5/405 (2)
1	367	349	113/349 (68)
2	568	531	192/531 (78)
6	262	236	219/236 (93)

^a Cells of the genotype *ura3-52-cut site-URA3 pGAL10::HO* (tNR111) were induced as described in Materials and Methods. Samples were taken at 0, 1, 2, and 6 h. Samples taken at 0 to 2 h were from a time course different from that of samples taken at the 6-h time point.

^b A *Trp⁺* phenotype indicates the presence of the *GAL10::HO* plasmid.

FIG. 5. Kinetic analysis of *HO* induction of strain tNR111. DNA was extracted from samples taken during a galactose induction of tNR111, as described in Materials and Methods. The DNA was digested with *Bam*HI, and the fragments were separated on an 0.5% agarose gel and transferred to nitrocellulose membrane. The filter was hybridized to a 1.2-kb *Hind*III *URA3* fragment that was gel isolated and labeled with [γ -³²P]dCTP by nick translation. P, Parental restriction fragment, C' and C'', fragments produced from P by *HO* cutting; D, deleted product.

FIG. 6. Kinetic analysis of early events during HO induction of strain tNR111. DNA was extracted from samples taken at 5-min intervals during a galactose induction of tNR111 as described in Materials and Methods. The DNA was digested with *Bam*HI, and the fragments were separated on an 0.5% agarose gel and transferred to nitrocellulose membrane. The filter was hybridized to plasmid YIp5 (*URA3*/pBR322) that was labeled with [γ - ^{32} P]dCTP by nick translation. The bands are labeled as in Fig. 5 with the exception of the pGALHO bands, which resulted from hybridization of the pBR322 sequences in the probe to plasmid pJH283.

course. The DNA samples were digested with *Bam*HI, which lacks recognition sites in either *URA3*, Ty, or the integrated sequences, and probed with a gel-isolated, nick-translated *Hind*III *URA3* fragment. The 14.5-kb uninduced parental band, P, is shown in lane 1 of Fig. 5. By 30 min after induction (Fig. 5, lane 2), two smaller bands, C' and C'', were visible. An HO cut divides the region into a 1.7-kb band containing *URA3* (C') and a 12.8-kb band representing the *ura3-52* portion (Fig. 5, C''). The 11.5-kb product band, D, indicative of the deletion-repair event, appeared at 1.5 h (Fig. 5, lane 4). The 1-h delay between the first appearance of HO-cut molecules and the first evidence of the deletion product seems surprisingly long. A similar delay, however, has been noted in a physical analysis of mating-type switching (4). An experiment addressing this point is discussed below. The majority of material had reacted by 5 h, and most of the unreacted material is presumed to represent the 10 to 20% of the population which had lost the *GAL10::HO* plasmid. It may be noted that there is no detectable evidence of a 5.5-kb band, which would result from formation of the *Ura*⁺ recombinant product; this is consistent with the genetic results.

No evidence is seen for either degradation or excision of sequences internal to the *URA3* duplication. The plasmid sequences integrated between the two *URA3* regions were clearly lost during the deletion-repair event, but neither the genetic or molecular data seem to provide a clue to the mechanism of removal. There was no obvious evidence of degradation, such as a ladder of partially digested molecules; if sequential degradation occurred, it must have happened extremely quickly, possibly coincident with strand exchange and repair. In one experiment, time points were taken at 5-min intervals during the first 3 h (Fig. 6). No evidence for degradation was observed within these time intervals. Another possibility is that a portion of the molecule was excised as an intact fragment. Given that HO cutting occurs early in

FIG. 7. Kinetic analysis of HO induction of strain tNR111 in the presence of cycloheximide. DNA was extracted from samples taken during a galactose induction of tNR111 as described in Materials and Methods. Cycloheximide (100 μ g/ml) was added 45 min after galactose induction (between lanes 2 and 3). The DNA was digested with *Bam*HI, and the fragments were separated on an 0.5% agarose gel and transferred to nitrocellulose membrane. The filter was hybridized to a 1.2-kb *Hind*III *URA3* fragment that was gel-isolated and labeled with [γ - ^{32} P]dCTP by nick translation. The bands are labeled as in Fig. 5.

the reaction, a second set of precise cuts, at the junctions between *ura3-52* or *URA3*, and the intervening pUC9 sequences might produce two fragments of approximately 2.6 kb and 50 bp containing DNA from the internal sequences (Fig. 3); although a 50-bp fragment would not have been resolved, a 2.6-kb band might have been detectable but was not visible on the gel (Fig. 6).

Completion of HO-induced recombination is inhibited by cycloheximide. The protein synthesis inhibitor cycloheximide was shown by Hereford and Hartwell (12) to prevent the initiation of chromosomal replication but not to affect DNA polymerization per se. It is not known whether new protein synthesis is needed to initiate DNA repair synthesis, although the induction of several genes involved in radiation repair has been shown to occur after DNA damage is generated (2, 3, 41). We were interested in whether a deletion-repair event could be completed in the presence of cycloheximide. A time course similar to the one described above was performed, this time by adding 100 mg of cycloheximide per ml at 45 min, when HO-induced cutting was already evident but before physical appearance of the restriction fragment indicative of the deletion. Although a small amount of product band was produced, it remained constant from 1.5 to 5 h, suggesting that a minor proportion of cells were able to initiate a recombination event before protein synthesis was stopped (Fig. 7). The most striking observation was that after HO endonuclease cutting was initiated, the HO-cut-specific molecules appeared to be completely stable throughout the 5 h and showed no evidence of degradation.

DISCUSSION

We have observed a novel recombinational repair event initiated by HO endonuclease. When the recognition site for the enzyme was embedded in non-*S. cerevisiae* sequences and placed between two regions of homology, recombination

was efficiently stimulated between the homologous flanking regions and was apparently always associated with concomitant loss of the internal sequences to yield a deletion, the product of an intrachromosomal exchange between direct repeats. When the 117-bp HO cut site replaced an intact *MAT* gene, this was the only detectable event. The HO cut could be located as far as 6.5 kb from or as close as 50 bp to a region involved in the deletion-repair event with no obvious loss of efficiency. The event is *RAD52* dependent and apparently also dependent on new protein synthesis for its completion. This HO-induced recombinational repair event is not specific to the *URA3* gene; similar events have been observed when *MAT* was placed between two *LEU2* genes (N. Rudin, Ph.D. thesis, Brandeis University, 1987). Small deletions, spanning the HO cut site, such as were observed by Weiffenbach and Haber (50) and Klar et al. (18) were not detected in this system.

The deletion-repair event is a slow process. In an effort to determine the intermediate steps involved in this HO-initiated recombination event, a kinetic analysis of the deletion-repair event has been carried out. The 1-h delay observed between the first appearance of the cut molecule and the first evidence of the deleted product is similar to what has been observed for normal *MAT* switching (4) as well as in a *LACZ* duplication system that we have developed (Rudin and Haber, manuscript in preparation). One explanation for this delay is that induction of protein synthesis is required to repair the cut and/or complete any associated recombination events. This possibility is supported by the observation that when cycloheximide was added after HO cutting had occurred, a large proportion of the cut molecules failed to complete the recombination event. Although a small amount of product was formed, the majority of HO-cut molecules persisted throughout the 5-h time course as stable fragments. We recognize that cycloheximide may have pleiotropic effects and that a detailed investigation of this phenomenon is needed.

Possible mechanisms for deletion-repair. In order for the deletion-repair event to be completed, the interacting homologous sequences must efficiently locate each other and the sequences internal to the duplication must be removed. These partial reactions may involve a common mechanism. Since the plasmid sequences originally integrated between the *URA3* genes carry no yeast plasmid maintenance sequences, it is impossible to determine genetically whether the event is initially reciprocal; the fate of the internal sequences can be investigated only by physical means. There are two obvious possibilities: either processive nucleolytic degradation removes the HO-cut DNA, exposing homology, or this DNA is initially excised as two intact fragments after pairing occurs between the homologous flanking regions. We have found no direct evidence to support either possibility, even during a time course in which samples were taken only 5 min apart. This result is similar to what has been seen in the normal mating-type switching system (4); the mechanism for removal of the heterologous sequences remains undefined.

In light of the fact that HO-cut fragments seem quite stable in the absence of a continuation of the recombination event, it seems unlikely that the ends are degraded before recombination. In addition, unrestrained exonuclease activity might be expected to produce a different spectrum of events when the cut site is located next to *ura3-52* than when it is close to *URA3*. If degradation were to proceed through the Ty element and repair were to take place in the extreme 5' region, many more *Ura*⁺ cells might be expected than the

1% that were observed. In either case, degradation beyond *URA3* sequences should lead to cell death, since no homology would be available from which to repair the DSB. In fact, the efficiency of repair appeared similar for the different orientations, as judged by tetrad viability, and the vast majority of events yielded *Ura*⁻ cells. These conclusions are similar to those reached by Struhl (47) from investigations of the frequency and structure of plasmid integrations produced by restriction enzyme cuts very close to or within nonhomologous DNA inserted into the sequences that permit homologous integrative recombination between a plasmid and a chromosome. Struhl suggests that neither polymerase or exonuclease activities are rate-limiting steps in DSB repair and that degradation may be coupled to initiation of recombination.

In light of this, we must consider another possible mechanism which is derived from work by Stahl et al. (45) on the recBC system in phage λ . The creation of a DSB in phage λ in vivo with *EcoRI* leads to the stimulation of events that are best explained by the entry of the recBC enzyme complex onto the DNA at the site of the break and its subsequent movement down the DNA. As the enzyme travels down the DNA, local unwinding might generate single-stranded regions that may participate in pairing, thereby facilitating recombination (49). A similar scenario in the yeast system described here might be imagined whereby a DSB between regions of homology might be recognized by a recBC-like complex as an entry point for the enzyme(s) to travel down intact DNA until homology is found.

Since the repair reaction seems to occur over a relatively long time span, either the two ends of the break or the homologous regions that ultimately recombine must remain in close proximity. It is possible that HO endonuclease remains temporarily attached to both ends of the break or that the *URA3* regions are synapsed even before the cut. Higher-order chromosome structure might also play a role in maintaining the apparent stability of the recombination complex.

A 117-bp fragment containing the *MATa* cut site efficiently stimulates deletion-repair, while a 20-bp synthetic cut site shows markedly reduced HO cutting. A fragment as small as 117 bp, containing the HO recognition site, behaved in the same way as an intact *MAT* locus in stimulating the deletion-repair event. This work provides the first physical evidence that the 117-bp *BglIII-HincII MATa* fragment sustains a DSB in vivo, which is in agreement with the results observed in vitro (21, 22), and in vitro and in vivo for the intact *MAT* locus (4, 46, 51). We also found that a 20-bp synthetic cutting site, which is cut inefficiently in vitro (31), is dramatically less efficient in stimulating deletion-repair events in vivo. This suggests that the assay of cutting sites in vitro may not accurately reflect their capacity to act as substrates in vivo. One important distinction to be noted here is that the in vitro assays were carried out with HO endonuclease purified from *E. coli*. It is possible that binding of HO endonuclease to its recognition site in vivo depends on the participation of other yeast proteins and that the requirements for binding and cutting in vitro are less stringent. This possibility is currently being investigated.

ACKNOWLEDGMENTS

We are grateful to Fred Heffron and Rich Kostriken for providing plasmids containing small HO endonuclease recognition sites and to Fred Heffron and Ira Herskowitz for providing *GAL10::HO*-containing plasmids. We thank Michael Esposito, Michael Resnick,

Rodney Rothstein, and Michael Rosbash for suggestions concerning the manuscript.

This work was supported by Public Health Service grant GM20056 from the National Institutes of Health and by National Science Foundation grant DCB8409086. N.R. was a predoctoral trainee of training grant GM07122.

LITERATURE CITED

1. Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis. 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. *Gene* 8:17-24.
2. Budd, M., and R. K. Mortimer. 1984. The effect of cycloheximide on repair in a temperature conditional radiation sensitive mutant of *Saccharomyces cerevisiae*. *Radiat. Res.* 99:582-590.
3. Cole, G. M., D. Schild, S. T. Lovett, and R. K. Mortimer. 1987. Regulation of *RAD54* and *RAD52-LACZ* gene fusions in *Saccharomyces cerevisiae* in response to DNA damage. *Mol. Cell. Biol.* 7:1078-1084.
4. Connolly, B., C. I. White, and J. E. Haber. 1988. Physical monitoring of mating type switching in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 8:2342-2349.
5. Dunn, B., P. Szauter, M. L. Pardue, and J. W. Szostak. 1984. Transfer of yeast telomeres to linear plasmids by recombination. *Cell* 39:191-201.
6. Game, J. C., T. J. Lamb, R. J. Braun, M. Resnick, and R. M. Roth. 1980. The role of *RAD* genes in meiotic recombination in yeast. *Genetics* 94:51-68.
7. Greider, C. W., and E. H. Blackburn. 1985. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 43:405-413.
8. Guarente, L., R. R. Yocum, and P. Gifford. 1982. A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. *Proc. Natl. Acad. Sci. USA* 79:7410-7414.
9. Haber, J. E. 1983. Mating type genes of *Saccharomyces cerevisiae*, p. 559-650. In J. A. Shapiro (ed.), *Mobile genetic elements*. Academic Press, Inc., New York.
10. Haber, J. E., and M. Hearn. 1985. *RAD52*-independent mitotic gene conversion in *Saccharomyces cerevisiae* frequently results in chromosome loss. *Genetics* 111:7-22.
11. Haber, J. E., and P. Thorburn. 1984. Healing of broken linear dicentric chromosomes in yeast. *Genetics* 106:207-226.
12. Hereford, L. M., and L. H. Hartwell. 1973. The role of protein synthesis in the replication of yeast DNA. *Nature (London)* New Biol. 244:129-131.
13. Hicks, J. B., and I. Herskowitz. 1976. Interconversion of yeast mating type. I. Direct observation of the action of the homothallism (*HO*) gene. *Genetics* 83:245-258.
14. Ito, H., Y. Fukada, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153:163-168.
15. Jackson, J. A., and G. R. Fink. 1981. Gene conversion between duplicated genetic elements in yeast. *Nature (London)* 292:306-311.
16. Klar, A. J. S., J. B. Hicks, and J. M. Strathern. 1982. Directionality of yeast mating-type interconversion. *Cell* 28:551-561.
17. Klar, A. J. S., and J. N. Strathern. 1984. Resolution of recombination intermediates generated during yeast mating type switching. *Nature (London)* 310:744-748.
18. Klar, A. J. S., J. N. Strathern, and J. A. Abraham. 1984. Involvement of double-strand chromosomal breaks for mating-type switching in *Saccharomyces cerevisiae*. *Cold Spring Harbor Symp. Quant. Biol.* 49:77-88.
19. Klar, A. J. S., J. N. Strathern, and J. B. Hicks. 1984. Developmental pathways in yeast, p. 151-195. In R. Resnick and L. Shapiro (ed.), *Microbial development*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. Kolodkin, A. L., A. J. S. Klar, and F. Stahl. 1986. Double-strand breaks can initiate meiotic recombination in *Saccharomyces cerevisiae*. *Cell* 46:733-740.
21. Kostriken, R., and F. Heffron. 1984. The product of the *HO* gene is a nuclease: purification and characterization of the enzyme. *Cold Spring Harbor Symp. Quant. Biol.* 49:89-104.
22. Kostriken, R., J. N. Strathern, A. J. S. Klar, J. B. Hicks, and F. Heffron. 1983. A site-specific endonuclease essential for mating-type switching in *Saccharomyces cerevisiae*. *Cell* 35:167-174.
23. Kunes, S., D. Botstein, and M. S. Fox. 1985. Transformation of yeast with linearized plasmid DNA: formation of inverted dimers and recombinant plasmid products. *J. Mol. Biol.* 184:375-387.
24. Lichten, M., R. H. Borts, and J. E. Haber. 1987. Meiotic gene conversion and crossing over between dispersed homologous sequences occurs frequently in *Saccharomyces cerevisiae*. *Genetics* 117:233-246.
25. Malone, R. E., and R. E. Esposito. 1980. The *RAD52* gene is required for homothallic interconversion of mating type 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. McCusker, J. H., and J. E. Haber. 1981. Evidence of chromosomal breaks near the mating-type locus of *Saccharomyces cerevisiae* that accompany *MAT α × MAT α* matings. *Genetics* 99:383-403.
28. Mortimer, R. K., R. Contopoulou, and D. Schild. 1981. Mitotic chromosome loss in a radiation-sensitive strain of the yeast *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 78:5778-5782.
29. Nasmyth, K. A. 1983. Molecular genetics of yeast mating type. *Annu. Rev. Genet.* 16:439-500.
30. Nasmyth, K. A. 1983. Molecular analysis of a cell lineage. *Nature (London)* 302:670-676.
31. Nickoloff, J. A., E. Y. Chen, and F. Heffron. 1986. A 24-base-pair DNA sequence from the *MAT* locus stimulates intergenic recombination in yeast. *Proc. Natl. Acad. Sci. USA* 83:7831-7835.
32. 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.
33. 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.
34. Oshima, T., and I. Takano. 1980. Mutants showing heterothallism from a homothallic strain of *Saccharomyces cerevisiae*. *Genetics* 94:841-857.
35. Prakash, S. L., L. Prakash, W. Burke, and B. A. Montelone. 1980. Effects of the *RAD52* gene on recombination in *Saccharomyces cerevisiae*. *Genetics* 94:31-50.
36. Resnick, M. A. 1975. The repair of double strand breaks in DNA: a model involving recombination. *J. Theor. Biol.* 59:97-106.
37. 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.
38. Rose, M., and F. Winston. 1984. Identification of a Ty insertion within the coding sequence of the *Saccharomyces cerevisiae* *URA3* gene. *Mol. Gen. Genet.* 193:557-560.
39. Rothstein, R. J. 1983. One-step gene disruption in yeast. *Methods Enzymol.* 101:202-211.
40. Rothstein, R. J., C. Helms, and N. Rosenberg. 1987. Concerted deletions and inversions are caused by mitotic recombination between delta sequences in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 7:1198-1207.
41. Ruby, S., and J. Szostak. 1985. Specific *Saccharomyces cerevisiae* genes are expressed in response to DNA-damaging agents. *Mol. Cell. Biol.* 5:75-84.
42. 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.
43. Sherman, F., G. R. Fink, and J. B. Hicks. 1986. *Methods in yeast genetics: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*

- 98:503–517.
45. Stahl, M. M., I. Kobayashi, F. W. Stahl, and S. K. Huntington. 1983. Activation of chi, a recombinator, by the action of an endonuclease at a distant site. *Proc. Natl. Acad. Sci. USA* **80**:2310–2313.
46. 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-stranded cut in the *MAT* locus *Cell* **31**:183–192.
47. Struhl, K. 1987. Effect of deletion and insertion on double-strand-break repair in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7**:1300–1303.
48. Tatchell, K., K. A. Nasmyth, B. D. Hall, C. Astell, and M. Smith. 1981. *In vitro* mutation analysis of the mating-type locus in yeast. *Cell* **27**:25–35.
49. Taylor, A. F., D. W. Schultz, A. S. Ponticelli, and G. R. Smith. 1985. *RecBC* enzyme nicking at chi sites during unwinding: location and orientation-dependence of the cutting. *Cell* **41**:153–163.
50. 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.
51. Weiffenbach, B., D. T. Rogers, J. Haber, M. Zoller, D. W. Russell, and M. S. 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.