MOLECULAR AND CELLULAR BIOLOGY, Oct. 1986, p. 3401-3409
0270-7306/86/103401-09$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 6, No. 10

Repair of Heteroduplex Plasmid DNA after Transformation into *Saccharomyces cerevisiae*

DOUGLAS K. BISHOP[1,2] AND RICHARD D. KOLODNER[1,3]*

Laboratory of Molecular Genetics, Dana Farber Cancer Institute,[1] and Department of Biological Chemistry, Harvard Medical School,[3] Boston, Massachusetts 02115, and Department of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts 02138[2]

Received 3 January 1986/Accepted 17 June 1986

Purified heteroduplex plasmid DNAs containing 8- or 12-base-pair insertion mismatches or AC or CT substitution mismatches were used to transform *Saccharomyces cerevisiae*. Two insertion mismatches, separated by 943 base pairs, were repaired independently of each other at least 55% of the time. This suggested that repair tracts were frequently shorter than 1 kilobase. The two insertion mismatches were repaired with different efficiencies. Comparison of the repair efficiency of one mismatched site with or without an adjacent mismatch suggests that mismatches promote their own repair and can influence the repair of neighboring mismatches. When two different plasmids containing single-insertion mismatches were transformed into *S. cerevisiae* cells, a slight preference towards insertion was detected among repair products of one of the two plasmids, while no repair preference was detected among transformants with the second plasmid.

Repair of mismatched nucleotides plays a role in determining spontaneous mutation rates, in protecting cells from DNA damage, and in general genetic recombination (7–11, 21–23). Mismatches result during genetic recombination when heteroduplex DNA is formed from homologous regions of DNA that differ in their nucleotide sequence. Mismatch repair, or failure of repair, during recombination has been used to explain such phenomena as gene conversion, postmeiotic segregation, localized negative interference, and map expansion (7, 25). However, the more recently proposed double-strand break-repair model suggests that these phenomena can be explained without invoking repair of mismatched nucleotides (29). A detailed understanding of the nature of mismatch repair reactions should therefore provide insight into the role of mismatch repair in recombination in *Saccharomyces cerevisiae*.

In *Escherichia coli*, transformation of purified phage or plasmid heteroduplex DNAs into cells, followed by analysis of clonally derived DNA from individual transformants, has proven particularly useful in the study of mismatch repair (4, 5, 16, 31). In these studies, phage or plasmid DNA obtained from individual transformants provides a record of a single molecular event that occurred following entry of a heteroduplex plasmid DNA molecule into a cell. In this study, we used the same approach to study mismatch repair in *S. cerevisiae*. This method has two major advantages over less direct genetic techniques. First, the products of a large number of repair reactions having identical substrates can be analyzed. Second, the existence of each heteroduplex substrate is known and need not be inferred from the assumptions of a particular recombination model. This last point is particularly important, since different recombination models attribute different roles for mismatches in recombination. For instance, the Aviemore model assumes that 6:2 and 2:6 meiotic segregations arise from repair of heteroduplex regions and that 5:3 and 3:5 segregations result from failure to repair heteroduplex regions (19). The double-strand break-repair model, however, suggests that 6:2 and 2:6 segregations can be accounted for by repair of double-strand gaps, a mechanism which requires no heteroduplex intermediate (and hence no repair) at the converted locus (29).

Here we demonstrate that mismatch repair occurs following transformation in *S. cerevisiae*, and in future communications we will describe the specificities of mismatch repair reactions and the effects of mutations that decrease the frequency of mismatch repair.

MATERIALS AND METHODS

Enzymes and chemicals. Restriction endonucleases and *Xba*I linkers having the sequence d(CTCTAGAG) were purchased from New England Biolabs, Beverley, Mass. [α-^{32}P]dATP, used in nick translations, was purchased from New England Nuclear Corp., Boston, Mass. T4 DNA ligase was prepared by an unpublished method similar to that of Tait et al. (30), and T4 polynucleotide kinase was prepared by the method of Panet et al. (20).

Strains and plasmids. The *S. cerevisiae* strains used in these studies were MP49-3B (*MAT***a** *ura3-52*; the gift of Monica Penn, Harvard University) and DB747 (*MAT***a** *his3-1d leu2-3 leu2-112 trp1-289 ura3-52*; the gift of David Botstein, Massachusetts Institute of Technology). The *Escherichia coli* strain used as the host for plasmid construction experiments was RDK1400 (28). pRDK70 is an 8.72-kilobase (kb) circular dimer derivative of pBR322 that contains a unique *Xba*I site (28). The *S. cerevisiae* plasmids used were all derivatives of YCp50 (see Fig. 1). YCp50 contains the yeast *ARS1* and *CEN4* sequences, which allow stable maintenance of the plasmid in yeast cells at about 1 copy per cell, and the *URA3* gene, which serves as a selectable marker (26). pRDK75 was constructed by digesting YCp50 with *Hin*dIII, filling in the resulting 5′ overhangs with reverse transcriptase, and inserting an 8-base-pair (bp) *XbaI* linker essentially as described previously (2, 12, 13). pRDK76 was constructed by digesting YCp50 with *Nru*I (which leaves blunt ends) and inserting an *Xba*I linker (2, 12, 28). The length of the insertions present in pRDK75 and pRDK76 were 12 and 8 bp, respectively; the difference is due to the fill-in step during the construction of pRDK75. pRDK77 was constructed by inserting the 1.95-kb *LEU2*-containing *Sal*I fragment obtained from pBD9 into the *Sal*I site of YCp50.

* Corresponding author.

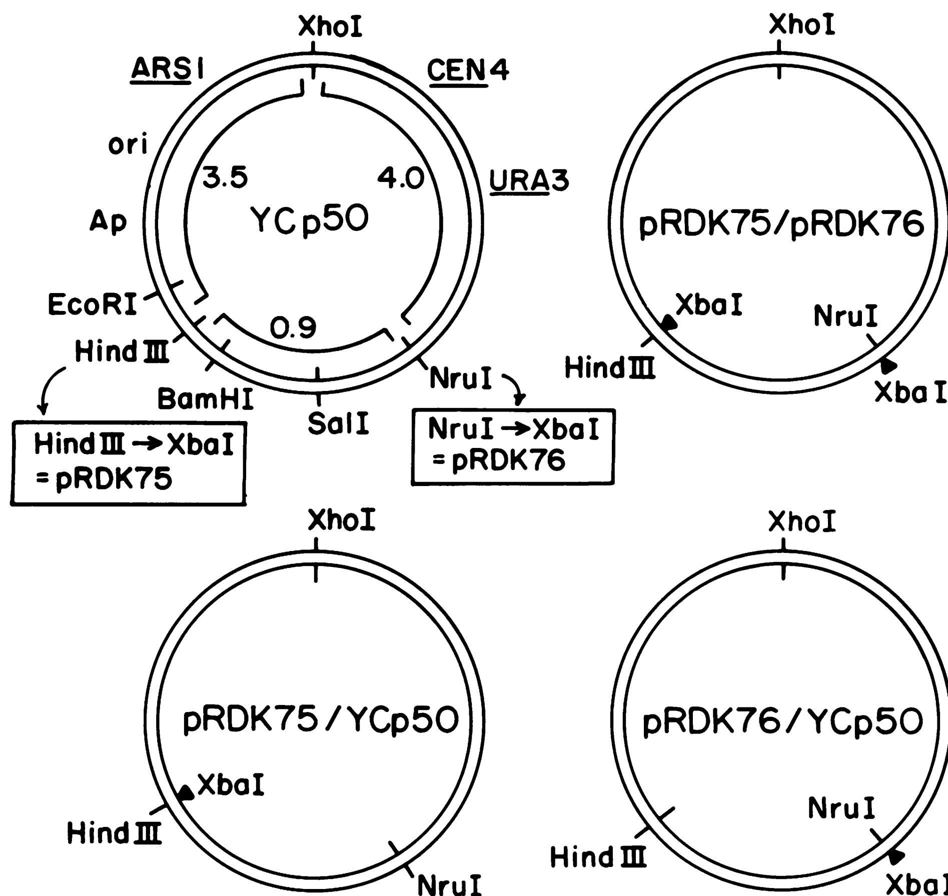


FIG. 1. Structure of heteroduplex substrates. The restriction map of YCp50 is not drawn to scale. YCp50 contains the *S. cerevisiae URA3*, *ARS1*, and *CEN4* sequences and the pBR322 tetracycline and ampicillin resistance genes. Distances between enzyme recognition sites are given in kilobase pairs. Each circle represents a single DNA strand. The triangles symbolize 8-bp (*Nru*I site) or 12-bp (*Hin*dIII site) insertions on one strand. The different heteroduplex preparations are equimolar mixtures of molecules having the configuration shown and molecules with the insertions at the same locations but on opposite strands.

pRDK78 was constructed by replacing the 0.38-kb *Eco*RI-*Bam*HI fragment of YCp50 with the 0.85-kb *Bgl*II-*Eco*RI fragment of A103p1, which contains the *TRP1* structural gene. pBD9 and A103p1 were provided by Barbara Dunn and Andrew Murray, respectively (Massachusetts General Hospital, Boston, Mass.). The methods used for the construction and analysis of plasmids and for the purification of plasmid DNA have been described (2, 12, 13).

Preparation of heteroduplex plasmid. We prepared heteroduplex plasmid DNA by two different methods. The first method has been described previously (2, 4; R. A. Fishel and R. Kolodner, submitted for publication). This method exploits the fact that a restriction endonuclease cleavage site is resistant to cleavage if it contains a mismatched nucleotide (4, 17). Briefly, mixtures of two linearized plasmid DNAs are denatured and then reannealed. The resulting DNA is circularized with T4 DNA ligase and then digested with a restriction endonuclease which cleaves only homoduplex DNA. Covalently closed circular heteroduplex DNA is then purified by equilibrium centrifugation in CsCl-ethidium bromide density gradients. The purity of these substrate DNA preparations was assessed by digesting them with an enzyme(s) capable of cleaving contaminating homoduplex but not heteroduplex DNA, followed by separation of digestion products by electrophoresis through 0.8% agarose gels (see Fig. 2). The heteroduplex preparations used in our studies were free of contaminating homoduplex plasmid (<5%). Some monomer-heteroduplex plasmid DNA containing single-strand interruptions (5 to 20%) was present in the substrate DNA preparations.

Our nomenclature for these types of heteroduplex DNA preparations separates the names of the two plasmids used to construct the heteroduplex DNA by a slash mark, e.g., pRDK75/pRDK76. The preparations are equimolar mixtures of duplexes containing the plus strand of one plasmid with the minus strand of the second and duplexes containing the opposite configuration of strands. To simplify the description of these experiments, insertion mismatches are designated A and B: A is the location of the *Hin*dIII site of YCp50, and B is the location of the *Nru*I site of YCp50. By this convention, pRDK75/pRDK76 has a 12-bp insertion mismatch at site A and an 8-bp insertion mismatch at site B. The three types of insertion mismatch-containing heteroduplex substrates used in this study are diagrammed in Fig. 1.

The second type of heteroduplex substrate was constructed by inserting oligonucleotide duplexes containing single-base-pair substitution mismatches into YCp50. The synthetic oligonucleotides were the gift of Claudia Muster-Nassal of this laboratory, who suggested this method for

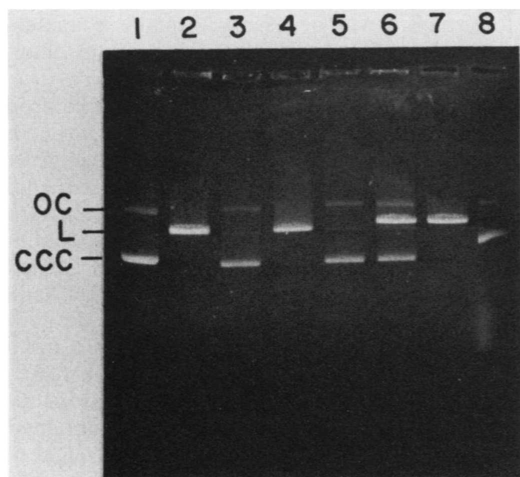
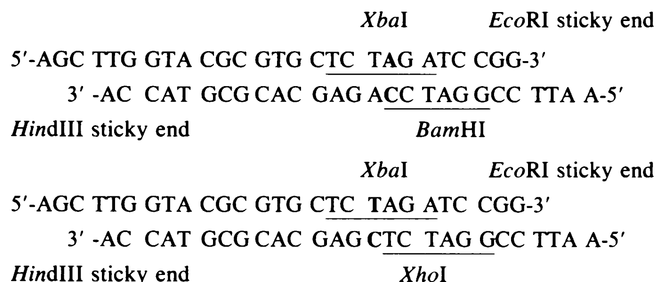


FIG. 2. Purity of pRDK75/pRDK76 preparation. Undigested pRDK75 (lane 1) and pRDK75 digested with *Xba*I (lane 2) were used as size standards for the various forms of pRDK75/pRDK76 DNA. Lane 3 is pRDK75/pRDK76 that was incubated under the same restriction endonuclease digestion conditions as the DNA in lane 5 except no enzyme was added. Lane 4 is pRDK75/pRDK76 digested with *Xho*I. Lane 5 is pRDK75/pRDK76 that was digested with *Xba*I to linearize any contaminating homoduplex DNA. Lane 6 is an *Xba*I digest of a mixture of pRDK75/pRDK76 and pRDK70 DNA that was performed to ensure that the resistance to cleavage with *Xba*I was not due to suboptimal reaction conditions. Lane 7 is an *Xba*I digest of pRDK70, and lane 8 is undigested pRDK70. The abbreviations used for the three monomer plasmid forms are: OC, open circular; L, linear; and CCC, covalently closed circular.

constructing heteroduplex DNA. The sequences of the oligonucleotide duplexes are:



*Xba*I *Eco*RI sticky end
5′-AGC TTG GTA CGC GTG CTC TAG ATC CGG-3′
3′ -AC CAT GCG CAC GAG ACC TAG GCC TTA A-5′
*Hin*dIII sticky end *Bam*HI

*Xba*I *Eco*RI sticky end
5′-AGC TTG GTA CGC GTG CTC TAG ATC CGG-3′
3′ -AC CAT GCG CAC GAG CTC TAG GCC TTA A-5′
*Hin*dIII sticky end *Xho*I

The mismatch-containing oligonucleotide duplexes were phosphorylated at their 5′ ends with T4 polynucleotide kinase (2) and then inserted into YCp50 by the following two-step ligation procedure. YCp50 was digested with *Eco*RI and *Hin*dIII, and the 25-bp *Eco*RI-*Hin*dIII fragment was removed by gel filtration on a column of Bio Gel P60 (BioRad Laboratories) run in 10 mM Tris hydrochloride (pH 8.0)–1 mM EDTA. The vector, at a concentration of 200 μg/ml, was mixed with a 50-fold molar excess of oligonucleotide, and the DNA was incubated with T4 DNA ligase at a concentration of 400 U/ml (13). The resulting high-molecular-weight DNA was then purified by phenol extraction and ethanol precipitation and digested with *Hin*dIII to produce monomer-length vector molecules containing a single mismatched oligonucleotide. The linear heteroduplex monomer DNA was then incubated with 5 U of T4 DNA ligase per ml at a DNA concentration of 2 μg/ml to circularize the plasmid DNA. The resulting covalently closed circular DNA was purified by equilibrium centrifugation in CsCl-ethidium bromide density gradients (12, 15). This two-step ligation procedure was carried out to ensure that the resulting covalently closed circular heteroduplex plasmid DNA contained only a single oligonucleotide duplex insertion.

Transformation. Heteroduplex plasmids were introduced into spheroplasted yeast cells essentially as described previously (24). A 50-ng amount of heteroduplex DNA was mixed with 50 μg of salmon sperm DNA and with 5 μg of M13 replicative form I DNA. This mixture was used to transform spheroplasts prepared from 100 ml of log-phase cells that had been grown at 30°C in YPD medium with shaking until they reached an OD_{590} of 0.8 to 1.0. Carrier DNA was used to avoid cotransformation. The cotransformation frequency under these conditions was approximately 0.02. Transformation efficiencies were between 3×10^3 and 1×10^4 transformants per μg of heteroduplex DNA.

Analysis of plasmid DNA. Individual yeast transformants, picked with a pasteur pipette to ensure collection of the entire colony, were used to inoculate 3 ml of YPD medium, and the cultures were shaken at 30°C. DNA was prepared from 1.5 ml of an overnight culture by treating spheroplasts with alkali and sodium dodecyl sulfate and centrifugation as described previously (24); one-tenth of the total DNA was used for each analysis. Digestion of DNA with restriction endonucleases and agarose gel electrophoresis was done as described previously (2, 12). Southern blots were prepared on Gene Screen filters (New England Nuclear) by the procedure recommended by the manufacturer. Processing of blots and hybridizations were carried out by the method of Church (1). pBR322 [^{32}P]DNA prepared by nick translation was used as the hybridization probe (18); the specific activity was about 5×10^7 cpm/μg.

Calculations and statistics. Different repair parameters were calculated as follows. Efficiency of repair at site A: (I + II + III + IV + V + VI)/total; efficiency of repair at site B: (I + II + III + IV + VI + VII)/total; minimum frequency of independent repair: [2(III + IV) + V + VI + VII + VIII]/total; frequency of corepair: [(I + II) − (III + IV)]/total; frequency of double independent repair: [2(III + IV)]/total; and frequency of single independent repair: (V + VI + VII + VIII)/total. Roman numerals refer to the number of transformants in the respective classes shown in Table 1, and "total" is the total number of transformants.

Statistically significant differences were distinguished from sampling errors by either chi-squared analysis or (when sample sizes were small) Fisher exact tests (6). P values less than 0.05 were considered significant.

RESULTS

Experimental design. We have developed an experimental system in which heteroduplex plasmid DNAs containing mismatched nucleotides are transformed into mitotic *S. cerevisiae* spheroplasts and the fate of the plasmid DNA after subsequent mismatch correction is determined by restriction mapping. All of the substrates contained the *URA3* gene, which acts as a selectable marker. The *CEN4* and *ARS1* sequences were also present to allow replication and stable maintenance of the plasmids.

Five different substrates were used in the experiments described below. pRDK75/YCp50 (Fig. 1) contains a 12-nucleotide insertion/deletion mismatch at site A so that repair events that insert the 12 nucleotides create an *Xba*I site at site A and repair events that delete the 12 nucleotides create a *Hin*dIII site at site A. pRDK76/YCp50 contains an 8-nucleotide insertion/deletion mismatch at site B so that repair favoring insertion creates an *Xba*I site and repair favoring deletion creates an *Nru*I site (Fig. 1). pRDK75/

pRDK76 contains both the 12-bp insertion/deletion mismatch at site A and the 8-bp mismatch at site B. The two mismatched sites in pRDK75/pRDK76 are 943 bp apart (Fig. 1). The AC mispair-containing substrate (see Materials and Methods) was designed so that conversion of AC to AT will create an *Xba*I site and conversion of AC to GC will create a *Bam*HI site (see Materials and Methods). The TC mispair-containing substrate was designed so that conversion of TC to TA will yield an *Xba*I site and conversion of TC to GC will yield an *Xho*I site.

Individual substrates are used to transform spheroplasts prepared from mitotic cells, and Ura^+ transformants are selected. Total DNA is then purified from individual transformants. The structure of the plasmid DNA is then determined by Southern blotting of total DNA either directly or after digestion with appropriate restriction endonucleases. This analysis allows us to unambiguously determine the structure of the plasmid DNAs present in individual transformants and draw inferences about how the initial substrate DNAs were processed after transformation.

Characterization of the system. To obtain data on the repair of heteroduplex plasmids that can be interpreted unambiguously, the following conditions must be met: (i) cells must be transformed by only a single heteroduplex DNA molecule; (ii) the method used to detect the different possible repair products must be unbiased; and (iii) the frequency of intermolecular recombination must be low.

The first criterion, transformation by a single heteroduplex molecule, was met by using a large excess of carrier DNA. The frequency of cotransformation was determined by control experiments in which a mixture of 50 ng of pRDK75 and 50 ng of pRDK76 was used to transform MP49-3B. In two control transformations, 1 of 75 and 0 of 60 clones were found to contain both plasmids. Using this data, we estimate a cotransformation frequency of about 1%. This estimate should be raised to about 2% because transformants resulting from cotransformation with two copies of the same plasmid cannot be detected by this method.

One of the possible fates of a heteroduplex plasmid after transformation is replication prior to mismatch repair, which yields a transformant colony containing two different plasmid DNAs. To determine whether repair occurs at a given mismatched site, it is necessary to know the frequency with which stable transformants containing two unselected centromere plasmids are detected by Southern blotting. If the frequency is low, then the method will be unable to distinguish between repair and failure to detect one of the two products of semiconservative DNA replication. To estimate the efficiency of detection of unselected double genotype transformants, a mixture of pRDK77 and pRDK78 DNA was transformed into DB747 under conditions favoring cotransformation(1.0 μg of each plasmid, no carrier DNA). Transformants were selected for uracil prototrophy. The transformants were then picked and grown on YPD master plates and transferred by replica plating to medium lacking leucine and tryptophan to detect clones that had been transformed with both plasmids. These clones were then picked from the original YPD master plate and grown in liquid YPD medium. Plasmid DNA was prepared from these cultures and analyzed by the Southern blotting method. Eighty percent of the cotransformants contained both monomer plasmids as determined by the Southern blotting assay. Therefore, the physical analysis detects 80% of the transformants which are defined by genetic criteria as having originally contained two different plasmids. This estimate may be low, because transformants from the control experiment, for which it was necessary to make a master plate, were grown longer on nonselective medium than were transformants obtained with heteroduplex DNA.

Another possible complication is that after a heteroduplex plasmid DNA molecule enters a cell and replicates, the two resulting plasmids may recombine prior to segregation during the first cell division. The reported frequencies of intramolecular plasmid recombination events range from 0.1 to 1% per cell generation, with intermolecular recombination occurring at 100-fold-lower frequencies (3, 14). These observations suggest that recombination events after replication of heteroduplex plasmids are highly unlikely. Nevertheless, a control experiment was performed in which a mixture of pRDK75 and pRDK76 DNA was used to transform strain MP49-3B under conditions favoring cotransformation (see above). The structure of the plasmid DNAs obtained from the transformants was then analyzed. Seventy-eight percent of the transformants contained either pRDK75 or pRDK76, while 22% of the transformants contained a mixture of pRDK75 and pRDK76. No recombinant configurations were observed, confirming that intermolecular recombination events are unlikely to occur at significant frequencies under our experimental conditions.

Repair of a plasmid containing two insertion mismatches. Heteroduplex DNA substrate pRDK75/pRDK76 (Fig. 1) was transformed into MP49-3B in four independent experiments. DNA was isolated from 274 individual transformants, and the structure of the plasmid DNA molecules obtained was analyzed. Plasmid-specific sequences were detected by Southern blot hybridization with nick-translated pBR322 as a probe. Analysis of undigested DNA showed that all of the plasmids recovered were circular monomers. To distinguish the nine possible types of repair products that could have been formed by either mismatch repair or DNA replication, two restriction endonuclease digestions were performed on DNA from each transformant. In one reaction, DNA was digested with *Xho*I to linearize the plasmid and with *Xba*I to cleave the DNA at sites having a linker insertion mutation; in a second reaction the DNA was linearized with *Xho*I and digested with *Hin*dIII and *Nru*I to cleave the DNA at sites where no linker insertion is present.

The nine possible repair classes which can be distinguished with this scheme are diagrammed in Fig. 3. Restriction mapping analysis of 14 individual transformants representing six of the nine possible types of repair products is presented in Fig. 4. A tabulation of all the data is presented in Table 1. The first class of transformants (Fig. 4, transformants 1, 2, 5, and 13) yielded 4.4- and 4.0-kb bands on digestion with *Xho*I and *Xba*I and yielded 4.9- and 3.5-kb bands on digestion with *Xho*I, and *Hin*dIII, and *Nru*I. This is the digestion pattern expected if the pRDK75/pRDK76 heteroduplex had been converted to a pRDK76 monomer. A second class of transformants (Fig. 4, transformants 4, 6, and 7) yielded 4.9- and 3.5-kb fragments on digestion with *Xho*I and *Xba*I, while digestion with *Xho*I, *Hin*dIII, and *Nru*I gave 4.0- and 4.4-kb fragments. This digestion pattern is the same as that of a pRDK75 monomer. A third class of transformant (Fig. 4, transformants 9 and 10) yielded 4-, 3.5-, and 0.9-kb fragments after digestion with *Xho*I and *Xba*I and yielded an 8.4-kb fragment after digestion with *Xho*I, *Hin*dIII, and *Nru*I. This digestion pattern would be obtained from a monomer plasmid that has an *Xba*I linker insertion at both sites A and B. A fourth class of transformant (Fig. 3, transformant 14) yielded an 8.4-kb fragment after digestion with *Xho*I and *Xba*I and yielded 4-, 3.5-, and 0.9-kb fragments after digestion with *Xho*I, *Hin*dIII, and *Nru*I. This is

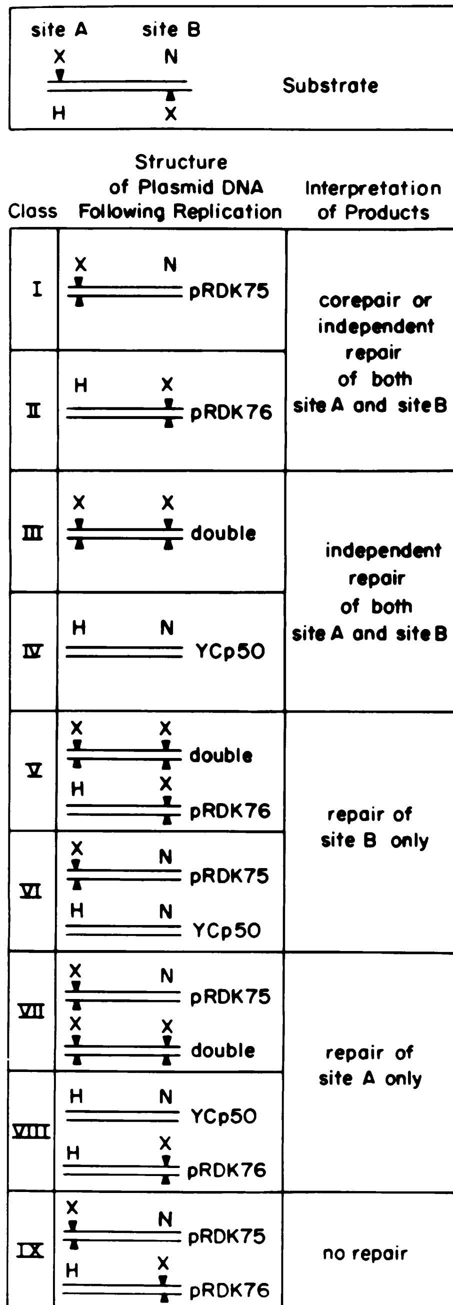


FIG. 3. Diagrams of possible pRDK75/pRDK76 repair products. Abbreviations: X, *Xba*I; H, *Hin*dIII; N, *Nru*I. The triangles represent 8-bp (site A) or 12-bp (site B) insertions. The plasmids shown would be obtained following the type of repair event indicated and subsequent DNA replication. The diagrams represent the plasmid DNA structure following linearization with *Xho*I. Classes I through IV are transformants with a single plasmid genotype, classes V through IX are transformants having a mixture of two plasmid genotypes. A description of the type of repair event(s) which could produce each class of product is given. For simplicity, the possible products of only one of the two configurations of pRDK75/pRDK76 are shown (see the legend to Fig. 1).

the digestion pattern of YCp50. The last two classes represented in Fig. 4 are transformants containing two different plasmids. Class five transformants (Fig. 4, transformants 3, 4, and 12) have restriction endonuclease cleavage patterns

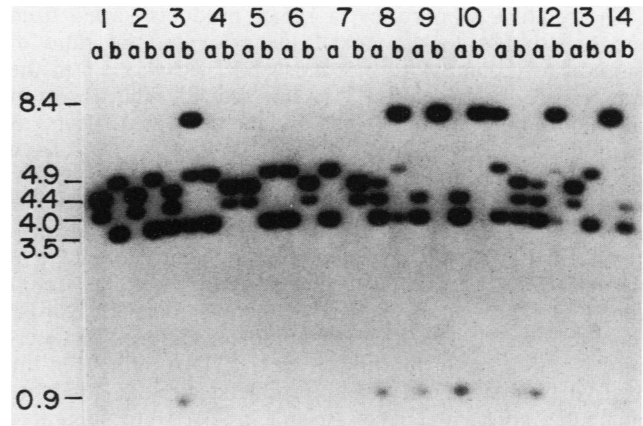


FIG. 4. Southern analysis of pRDK75/pRDK76 transformant DNA. The figure is an autoradiograph of a Southern blot analysis of plasmid DNA obtained from 14 pRDK75/pRDK76 transformants. The DNA from each transformant was digested with *Xho*I and *Xba*I (lanes a) or with *Xho*I, *Hin*dIII, and *Nru*I (lanes b). The hybridization probe used was ^{32}P-labeled pBR322 DNA, so that only plasmid sequences would be visualized. Sizes of the fragments are given (in kilobase pairs) at the left.

characteristic of a mixture of pRDK75 and the double mutant plasmid; class six (Fig. 4, transformant 11) has a digestion pattern which would be obtained from a mixture of YCp50 and pRDK75 DNA. In addition to the six classes of transformants represented on the Southern blot in Fig. 4, transformants having the cleavage patterns expected for two of the three remaining repair classes shown in Fig. 3 (classes VIII and IX) were observed among the 274 transformants analyzed. All 274 transformants analyzed were unambiguously assigned to one of the nine possible classes shown in Fig. 3.

The majority of the transformants fell into one of the four single genotype classes, with transformants having parental genotypes (pRDK75 or pRDK76) outnumbering those with recombinant genotypes (YCp50 or double mutant) in all four experiments (Table 1). The only class of products that was not observed was class VII (Fig. 3). If we assume that random strand loss occurs at low frequency (see discussion), the data in Table 1 may be used to calculate the efficiency of repair at each of the two mismatched sites (see Materials and Methods). The data show a significant difference in repair efficiency at sites A and B. Repair occurred at site A, the *Hin*dIII-*Xba*I mismatch, 76 to 91% of the time, while repair at site B, the *Nru*I-*Xba*I mismatch, occurred at a frequency of 98%. This difference was significant in each of the four experiments by Fisher's exact test ($P \leq 0.03$).

TABLE 1. Southern analysis of pRDK75/pRDK76 transformants[a]

Transformation no.	Single genotypes: Parental I	II	Recombinant III	IV	Double genotypes: V	VI	VII	VIII	IX	No. of transformants analyzed
1	24	19	9	5	5	11	0	0	0	73
2	12	11	11	6	5	8	0	1	1	55
3	18	22	7	6	4	2	0	0	1	60
4	30	38	5	4	7	1	0	0	1	86

[a] The transformant classes designated by roman numerals are the same as those shown in Fig. 3.

The relative proportions of repair products varied from one experiment to the next. For example, the ratio of parental to recombinant plasmids observed was 3.1 in the first transformation and 1.3 in the second. The observed variation cannot be explained by ramdom fluctuation, as determined by chi-squared analysis ($P = 0.0008$). DNA from the same heteroduplex preparation was used in the first two transformations and yielded somewhat different results. The only obvious difference in the DNA used in the second transformation was that it contained a higher percentage of circular DNA containing a single-strand break, as determined by electrophoresis of DNA samples-through agarose gels (20% versus 5% in the first transformation). To determine whether the proportion of nicked DNA influenced the relative frequencies of repair products, circular substrate molecules containing single-strand breaks were separated from covalently closed substrate molecules by equilibrium centrifugation in a CsCl-ethidium bromide density gradient and transformed into MP49-3B separately. Thirty transformants from each transformation were analyzed. The ratio of parental to recombinant plasmids was 3.5 for nicked substrate and 2.7 for covalently closed substrate; this difference was not statistically significant by chi-squared analysis. Therefore, the presence of single-strand breaks in the substrate molecules did not appear to affect the relative frequencies of repair products. Possible explanations for the variation observed in these experiments are presented in the Discussion.

Effect of an adjacent mismatch on repair efficiency. One possible explanation for the higher frequency of parental than recombinant plasmids among pRDK75/pRDK76 transformants is that adjacent mismatched sites may sometimes be repaired in a single concerted event. This type of repair, called corepair, has been observed in similar experiments with *E. coli* (31). To test this possibility, two heteroduplex preparations having single mismatched insertion mutations at either site A (pRDK75/YCp50) or B (pRDK76/YCp50) were constructed and transformed into MP49-3B. Repair of single-site heteroduplexes can be studied in this system if strand loss occurs at a low frequency (see Discussion). The efficiency of apparent repair at each single mismatched site was compared with that observed with the pRDK75/pRDK76 heteroduplex. When repair of pRDK75/pRDK76 was compared with repair of pRDK75/YCp50, a decrease in the apparent repair efficiency was observed at site A, and this decrease approached significance by Fisher's exact test ($P = 0.06$) (Table 2). A comparison of the data obtained with pRDK75/pRDK76 and with pRDK76/YCp50 revealed that apparent repair at site B was significantly less efficient in the absence of a mismatch at site A ($P = 0.008$). An additional experiment with pRDK76/YCp50 was performed (Table 2, line 5); while pRDK75/pRDK76 was not transformed in the same experiment, the results indicate that the reduction in apparent repair efficiency observed in the earlier transformation was reproducible.

Directionality of repair. To determine whether the apparent repair of mismatched insertion mutations favored incorporation or removal of the insertion, data from the experiments with pRDK75/YCp50 and pRDK76/YCp50 were analyzed by the binomial distribution test. In one experiment with the pRDK75/YCp50 heteroduplex, 62 of 96 repaired plasmids were repaired in favor of insertion. This result differs significantly from the perfectly random frequency of 0.5 ($P = 0.003$) and indicates a small bias in favor of insertion. The data from two experiments in which pRDK76/YCp50 transformants were analyzed did not reveal a statistically significant repair preference ($P \geq 0.09$). In addition, the deviation from parity was not significant ($P \geq 0.07$) when the same analysis was applied to the independent repair products obtained from transformation with pRDK75/pRDK76 (Fig. 3, classes III through VIII).

TABLE 2. Effect of adjacent mismatches on repair efficiency[a]

Site	Heteroduplex transformed	Adjacent mismatch present	Repair efficiency[b] (%)	No. of transformants analyzed
A	pRDK75/YCp50	No	81	119
	pRDK75/pRDK76	Yes	90	86
B	pRDK76/YCp50	No	89	85
	pRDK75/pRDK76	Yes	99	86
	pRDK76/YCp50[c]	No	80	117

[a] Analysis of the plasmid DNAs present in individual transformants was carried out essentially as described in the legend to Fig. 3 and Materials and Methods.
[b] Percentage of total transformants in which the specified site was repaired.
[c] The data in this row were obtained in a separate experiment.

Repair of single-base-pair mismatches. We also carried out experiments to determine the fate of heteroduplex plasmids containing single-base-pair mismatches following transformation into MP49-3B. YCp50 derivatives carrying AC and CT mismatches were constructed. The oligonucleotides used to construct these two heteroduplex plasmid DNAs code for different restriction endonuclease recognition sites (see Materials and Methods). An *Xba*I site will be created if the AC mismatch is repaired to AT following transformation into *S. cerevisiae*, and a *Bam*HI site will be created if the mismatch is converted to GC. Likewise, conversion of the CT mismatch to CG will yield an *Xho*I site, while conversion to AT will create an *Xba*I site. Plasmid DNA obtained from individual transformants was analyzed by digestion of the DNAs with appropriate enzymes, followed by electrophoresis on agarose gels and Southern blotting. Twenty-two transformants from plasmids originally containing a single AC mismatch were analyzed; 10 contained the GC product, 8 contained the AT product, and 4 contained both products. The results for the plasmid containing the TC mismatch were as follows: 14 contained the GC product, 5 contained the TA product, and 7 contained both products. Although the number of transformants analyzed was small, the results suggest that both a transition and a transversion mismatch can be repaired following transformation of the substrate DNA into MP49-3B.

DISCUSSION

This paper describes a method for studying mismatch repair by transforming heteroduplex plasmid DNA into *S. cerevisiae*. The results indicate that cells transformed with pRDK75/pRDK76 yield a variety of parental and recombinant forms. Ninety-eight percent of the transformants carry plasmid DNAs whose structure cannot be explained by simple replication of pRDK75/pRDK76 DNA molecules. We think the most likely explanation for these results is that the heteroduplex DNA is processed by an efficient mismatch correction system in *S. cerevisiae*. As outlined in the Results section, the possibility that the various products observed resulted from intermolecular recombination following DNA replication is unlikely. Transformants having recombinant genotypes, which accounted for 35% of the total (Fig. 3, classes III through VIII), are most easily explained by independent repair of one or both mismatched sites on pRDK75/pRDK76.

While the data in Table 1 provide clear evidence that repair of insertion mismatches occurs, the interpretation of transformants having a single plasmid genotype requires further comment. Wagner and Meselson (31), studying repair of bacteriophage lambda heteroduplexes, concluded that plaques that yielded a single phage genotype were not always the result of repair at every mismatched site. Instead, their data suggested that in some cases the products of one of the two DNA strands present in the heteroduplex substrate were not detected. In our case, loss of the information coded for by one of the two strands of a heteroduplex molecule could occur either before of after mismatch repair. This raises the possibility that pRDK75/pRDK76 transformants having a single genotype may result from random loss of a strand from transformants in which repair either did not occur or occurred only at one of the two mismatched sites. Similarly, strand loss might produce the distribution of plasmid types recovered from transformation with single-site heteroduplexes.

Loss of the information coded for by one of the two strands in a heteroduplex could occur either before or after the cell has an opportunity to repair the mismatched DNA. A strong argument can be made against the possibility that random strand loss occurs after the opportunity for repair. The distribution of products observed cannot be explained by random strand loss after repair or after the first round of posttransformation plasmid DNA synthesis. The strand loss hypothesis of Wagner and Meselson predicts that each of the four plasmid genotypes should occur at the same relative frequency among single genotype transformants and double genotype transformants. A cursory examination of the data in Table 1 indicates that more single genotype transformants have parental genotypes (classes I and II) than recombinant genotypes (classes III and IV), while plasmids having recombinant and parental genotypes occur at roughly equal frequency among double genotype transformants (classes V through VIII). The equal number of parental and recombinant plasmids among double genotype transformants is the result of the paucity of transformants in class IX, the only double genotype class which is not half-parental, half-recombinant (refer to Fig. 3). To determine whether the distribution of single genotype transformants could be explained by postrepair strand loss, we analyzed the data in Table 1 essentially as described by Wagner and Meselson. Because sample sizes were small, Fisher exact tests were used in place of the chi-squared tests (6). When the data from transformations 1 through 4 were analyzed, we found P values of 0.003, 0.11, 0.16, and 0.001, respectively. This analysis provides proof that strand loss does not account for the distribution of products observed in experiments 1 and 4. It does not follow that strand loss occurred in experiments 2 and 3, since the distribution of products observed could easily have been generated by repair. This type of analysis cannot eliminate the possibility that in some cases strand loss occurred prior to repair. Strand loss may have occurred, for example, during the transformation process as a result of cytoplasmic exonuclease activity. In addition, we cannot eliminate the possibility that in some cases the repaired strand is lost in an aborted repair event after initiation of repair. Such aborted repair events would resemble corepair. These latter two types of strand loss would lead to the recovery of an excess of parental single genotype transformants, and this will be discussed in more detail below.

If we assume that strand loss is rare in our system, then our data can be interpreted as follows: in Table 1, classes I and II result from either one concerted corepair event or two independent repair events, classes III and IV result from two independent repair events, and classes V through VIII result from a single independent repair event. The data presented above (Table 2 and text) indicate that single insertion/deletion mispairs and single-base-substitution mispairs were repaired efficiently and at near parity. The observation (Table 2) that a mismatch at site B was repaired more efficiently in the presence of a mismatch at site A suggests that the two mismatches present in pRDK75/pRDK76 can be corepaired. This observation is consistent with the observation of Fogel and his colleagues that adjacent heteroalleles can influence the ratio of 6:2 to 5:3 segregations during meiosis in *S. cerevisiae* (7, 8).

Since no bias towards insertion or deletion products was observed among independent repair products of pRDK75/pRDK76 (see above), the assumption may be made that about half of the products produced by two independent repair events on the same plasmid DNA molecule will have the same restriction endonuclease cleavage pattern as products produced by corepair (either pRDK75 or pRDK76). With this assumption, the frequency of double independent repair can be calculated as twice the frequency of obtaining transformants that contained a recombinant plasmid. Similarly, the frequency of corepair can be calculated as the difference between the frequency of transformants containing a single parental plasmid and the frequency of those containing a recombinant plasmid. If the data in Table 1 are analyzed in this way, 44% of the products were formed by corepair, 39% of the products were formed by two independent repair events, 16% of the products were formed by a single independent repair event, and in 1% of the transformants no repair occurred (the formulas used for these calculations are given in Materials and Methods). The assumption used in estimating the amount of corepair is valid only if double independent repair events involving excision on opposite strands of a heteroduplex plasmid molecule are as likely to produce functional plasmid molecules as are double independent repair events involving excision on the same strand. If excision tracts proceeding toward each other on opposite strands can result in the formation of double-strand breaks and these breaks then lead to plasmid loss, double independent repair events may result in more transformants having parental plasmid genotypes than recombinant ones. In this case, the estimate calculated above for the frequency of independent repair is low, while the estimate for corepair is high.

As discussed above, we cannot eliminate the possibility that strand loss may occur prior to or during heteroduplex repair. Because these two types of strand loss will only contribute to the single parental genotypes listed in Table 1, it is possible that all or part of the proportion of single parental genotype transformants that have been attributed to corepair may reflect this type of strand loss. Furthermore, the results obtained with single-site heteroduplexes would reflect a mixture of such strand loss and repair. While we consider it unlikely, if random strand loss occurs both before and after repair, then the products that we attribute to two independent repair events (Table 2, classes III and IV) would result from repair of one site followed by strand loss. We think that one way to resolve the effect of strand loss on the system described here would be to determine the effect of mismatch repair-defective mutations on the recovery of the different repair products. In collaboration with M. Williamson and S. Fogel (Berkeley, Calif.), we have been studying the effect of *pms1* mutations on this system (33). Our results (to be published elsewhere) suggest that most of

the single genotype transformants listed in Table 1 can be attributed to specific repair rather than strand loss.

The distribution of repair products observed provides information about the mechanism of mismatch repair in *S. cerevisiae*. As discussed above, regardless of the impact of strand loss, at least 55% of the products formed after transformation of the pRDK75/pRDK76 heteroduplex into mitotic cells involved independent repair. Therefore, it appears that excision-resynthesis tracts are frequently less than 943 bp long, the distance betwen the two mismatched sites. While the transformation process may perturb the normal mechanism of heteroduplex repair, our data are consistent with the results of mitotic and meiotic experiments on recombination in *S. cerevisiae*. Recombination products consistent with a mechanism involving both independent repair and corepair of heteroallelic mismatched sites have been observed in experiments on mitotic recombination in *S. cerevisiae* (10). In addition, the frequencies of meiotic conversion events at the *ARG4* locus are consistent with the frequency of independent repair we observed (7, 8). The *arg4-16* and *arg4-17* mutations located 214 bp apart were found to coconvert at a frequency of 75% (32). The *arg4-4* and *arg4-17* mutations coconverted at a frequency of 5%. The exact distance between *arg4-4* and *arg4-17* is not known but cannot be greater than 1,260 bp, the length of the *ARG4* gene. The reported coconversion frequencies of other *ARG4* intervals (all shorter than the *arg4-4* to *arg4-17* interval) are between 20 and 60%. If these data are interpreted in terms of the Aviemore model of recombination (19), mismatch correction excision-resynthesis tracts following meiotic recombination in *S. cerevisiae* are often longer than 214 bp and shorter than 1,000 bp. In *E. coli*, excision-resynthesis tracts have an estimated average length of 3,000 bp (31).

There was a significant variation in the frequencies of repair products in different experiments. The reason for this variation is unclear. One possibility is that variation in some aspect of the transformation procedure produces the observed variability. A second possibility is that the distance between the two mismatched sites in pRDK75/pRDK76 is similar to the average length of excision-resynthesis tracts. If this were the case, small variations in the average excision tract lengths or in the efficiency of initiation of repair may have produced the variation observed. Determining the effect of distance between mismatched sites on corepair frequency could provide insight into the source of this variability and into the average length of repair tracts.

The experiments described above investigated the repair of four different types of mismatches. The results indicate that mismatch correction is efficient during mitosis in *S. cerevisiae* and is therefore likely to process mismatched nucleotides formed during homologous recombination and by misincorporation during DNA replication. We are presently modifying the systems described here so that we can obtain additional information on both the specificity and mechanism of mismatch repair in *S. cerevisiae*.

ACKNOWLEDGMENTS

We thank Mary Ann Osley, Brian Hauge, Claudia Muster-Nassal, Alain Nicolas, and Rick Fishel for helpful discussions; David Norris and David Evans for critical reading of the manuscript; and Janet Anderson for advice on statistical analysis.

This work was supported by Public Health Service grant GM-29383 from the National Institutes of Health and American Cancer Society grant FRA-271 to R.D.K.

LITERATURE CITED

1. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81**:1991–1995.
2. **Doherty, M. J., P. T. Morrison, and R. Kolodner.** 1983. Genetic recombination of bacterial plasmid DNA: physical and genetic analysis of the products of plasmid recombination in *Escherichia coli*. J. Mol. Biol. **167**:539–560.
3. **Embretson, J. E., and D. M. Livingstone.** 1984. A plasmid model to study genetic recombination in yeast. Gene **29**:293–302.
4. **Fishel, R. A., and R. Kolodner.** 1983. Gene conversion in *Escherichia coli*: the identification of two repair pathways for mismatched nucleotides, p. 309–324. *In* E. C. Friedberg and B. A. Bridges (ed.), UCLA symposia on molecular and cellular biology, vol. 11. Alan R. Liss, Inc., New York.
5. **Fishel, R. A., E. C. Siegel, and R. Kolodner.** 1986. Gene conversion in *Escherichia coli*: resolution of heteroallelic mismatched nucleotides by co-repair. J. Mol. Biol. **188**:147–157.
6. **Fleiss, J. L.** 1981. Statistical methods for rates and proportions. John Wiley & Sons, Inc., New York.
7. **Fogel, S., R. K. Mortimer, and K. Lusnak.** 1982. Mechanisms of meiotic gene conversion or "wanderings on a foreign strand," p. 289–339. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, vol. 1. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Fogel, S., R. K. Mortimer, and K. Lusnak.** 1983. Meiotic gene conversion in yeast, p. 65–107. *In* J. F. T. Spencer, D. M. Spencer, and A. R. W. Smith (ed.), Molecular and experimental perspectives. Springer-Verlag, New York.
9. **Fogel, S., R. K. Mortimer, K. Lusnak, and V. Tavares.** 1979. Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43**:1325–1341.
10. **Golin, J. E., and M. S. Esposito.** 1981. Mitotic recombination: mismatch correction and replicational resolution of Holliday structures formed at the two strand stage in *Saccharomyces*. Mol. Gen. Genet. **183**:252–263.
11. **Holliday, R., P. J. Pukkila, J. M. Dickson, A. Spanos, and V. Murray.** 1979. Relationships between the correction of mismatched bases in DNA and mutability. Cold Spring Harbor Symp. Quant. Biol. **43**:1317–1323.
12. **James, A. A., P. T. Morrison, and R. Kolodner.** 1982. Genetic recombination of bacterial plasmid DNA: analysis of the effect of recombination-deficient mutations on plasmid recombination. J. Mol. Biol. **160**:411–430.
13. **James, A. A., P. T. Morrison, and R. Kolodner.** 1983. Isolation of genetic elements that increase frequencies of plasmid recombinants. Nature (London) **303**:256–259.
14. **Kiel, 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.
15. **Kolodner, R.** 1980. Genetic recombination of bacterial plasmid DNA: electron microscopic analysis of intramolecular recombination catalyzed *in vitro*. Proc. Natl. Acad. Sci. USA **77**:4847–4851.
16. **Kramer, B., W. Kramer, and H.-J. Fritz.** 1984. Different base/base mismatches are corrected with different efficiencies by the methy-directed DNA mismatch-repair system of *E. coli*. Cell **38**:879–887.
17. **Lu, A. L., S. Clark, and P. Modrich.** 1983. Methyl-directed repair of DNA base pair mismatches *in vitro*. Proc. Natl. Acad. Sci. USA **80**:4639–4643.
18. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 109–112. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Meselson, M. S., and C. M. Radding.** 1975. A general model for genetic recombination. Proc. Natl. Acad. Sci. USA **72**:358–361.
20. **Panet, A., J. H. van de Sande, P. C. Loewen, H. G. Khorana, A. J. Raae, J. R. Lillehaug, and K. Kleppe.** 1973. Physical characterization and simultaneous purification of bacteriophage T4 induced polynucleotide kinase, polynucleotide ligase and deoxyribonucleic acid polymerase. Biochemistry **12**:5045–5050.
21. **Pang, P. P., S.-P. Tsen, A. S. Lundberg, and G. C. Walker.** 1984. The mutH, mutL, mutS, and uvrD genes of *Salmonella*

typhimurium LT2. Cold Spring Harbor Symp. Quant. Biol. **49**:597–602.

22. **Radding, C. M.** 1978. Genetic recombination: strand transfer and mismatch repair. Annu. Rev. Biochem. **47**:847–880.
23. **Radman, M., G. Villani, S. Boiteux, A. R. Kinsella, B. W. Glickman, and S. Spadari.** 1979. Replicational fidelity: mechanisms of mutation avoidance and mutation fixation. Cold Spring Harbor Symp. Quant. Biol. **43**:937–946.
24. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1982. Methods in yeast genetics, p. 119B. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Stahl, F. W.** 1979. Genetic recombination: thinking about it in phage and fungi. W. H. Freeman & Co., San Francisco.
26. **Stinchcomb, D. T., C. Mann, and R. W. Davis.** 1982. Centromeric DNA from *Saccharomyces cerevisiae*. J. Mol. Biol. **158**:157–179.
27. **Symington, L., L. Fogarty, and R. Kolodner.** 1983. Genetic recombination of homologous plasmids catalyzed by cell-free extracts of yeast. Cell **35**:805–813.
28. **Symington, L. S., P. Morrison, and R. Kolodner.** 1985. Intramolecular recombination of linear plasmid DNA catalyzed by the *Escherichia coli* recE recombination system. J. Mol. Biol. **186**:515–525.
29. **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.
30. **Tait, R. C., R. L. Rodrigues, and R. W. West.** 1980. The rapid purification of T4 DNA ligase from a T4 *lig* lysogen. J. Biol. Chem. **255**:813–816.
31. **Wagner, R., and M. Meselson.** 1976. Repair tracts in mismatched DNA heteroduplexes. Proc. Natl. Acad. Sci. USA **73**:4135–4139.
32. **White, J. H., K. Lusnak, and S. Fogel.** 1985. Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate. Nature (London) **315**:350–352.
33. **Williamson, M. S., J. C. Game, and S. Fogel.** 1985. Meiotic gene conversion mutants in *Saccharomyces cerevisiae*. I. Isolation and characterization of *pms1-1* and *pms1-2*. Genetics **110**:609–646.