Copyright © 1988 by the Genetics Society of America

Double-Stranded Gap Repair of DNA by Gene Conversion in *Escherichia coli*

Ichizo Kobayashi*,† and Noriko Takahashi†

**Department of Bacteriology, Faculty of Medicine, University of Tokyo, Hongo, Tokyo 113, Japan, and †National Children's Medical Research Center, Taishido, Tokyo 154, Japan*

Manuscript received February 24, 1988
Accepted April 14, 1988

ABSTRACT

We demonstrated repair of a double-stranded DNA gap through gene conversion by a homologous DNA sequence in *Escherichia coli*. We made a double-stranded gap in one of the two regions of homology in an inverted orientation on a plasmid DNA molecule and introduced it into an *E. coli* strain which has the RecE system of recombination (genotype; *sbcA23 recB21 recC22*). We detected repair products by genetic selection. The repair products were those expected by the double-strand-gap repair model. Gene conversion was frequently accompanied by crossing over of the flanking sequences as in eukaryotes. This double-strand gap repair mechanism can explain plasmid recombination in the absence of an artificial double-stranded break reported in a companion study by Yamamoto *et al.*

THE rate of homologous recombination is not uniform along the DNA molecule. Analysis of the sites that enhance crossing over and gene conversion provides clues to the mechanisms of homologous recombination (for reviews, see STAHL (1979a,b)).

In the recombination system (Red) of the bacteriophage lambda, *cos* (cohesive end site), the site to be cleaved during packaging, turned out to be one of these special sites (STAHL, KOBAYASHI and STAHL 1985) [for reviews, see SMITH (1983) and STAHL (1986)]. The crossing-over events are concentrated at the ends of the lambda chromosome among the phage particles whose chromosomes have not been replicated (STAHL *et al.* 1974). The rate of crossing over is uniform along replicated lambda chromosomes. That *cos* sequence itself, rather than some other feature of the end region, is responsible for this bias was demonstrated through the use of lambda with translocated *cos* (STAHL, KOBAYASHI and STAHL 1982).

The "break-copy" model, as opposed to the break-join model or the copy-choice model, explains the *cos* action nicely as well as other features of the Red-mediated recombination of lambda (STAHL 1979a). The break-copy model supposes that recombination is initiated by the joining of one chromosome to another. The second chromosome is then copied by the replication fork created at the joint. When the replication fork reaches *cos*, the recombinant chromosome is completed and is packaged at that *cos*. When replication is limited, the exchange initiated at sites far from *cos* can not be completed by replication. Only the joinings which are close to *cos* and hence need only a short replication to reach *cos* can finish recombinant chromosomes.

The role of *cos* in this break-copy model is in packaging. The model predicts that a *cos* that cannot be used for packaging cannot enhance recombination. The critical experiments showed, however, that this is not the case (STAHL, KOBAYASHI and STAHL 1985; see also KOBAYASHI *et al.* 1983).

As an alternative to the break-copy model (though not a mutually exclusive one), a "double-stranded break" model for lambda recombination was proposed (STAHL, KOBAYASHI and STAHL 1985). In this model, the double-stranded break made at *cos* initiates, rather than terminates, recombination. Such double-stranded breaks at *cos* uncoupled from packaging was suggested from the analysis of *cos* action in another recombination pathway (KOBAYASHI *et al.* 1982, 1983, 1984). A single-stranded tail is produced by the 5′ to 3′ exonuclease activity of lambda exonuclease at the double-stranded end. The single strand is annealed with a homologous single strand with help from lambda beta protein (KMIEC and HOLLOMAN 1981; MUNIYAPPA and RADDING 1986). The tail of a rolling circle initiates recombination in a similar fashion all over the chromosome when recombination is allowed. This model explains the concentration of the exchanges near *cos* in the unreplicated chromosomes, their uniform distribution in the replicated chromosomes, the structure of the primary recombination products (WHITE and FOX 1974), the enzymatic activities of *exo* and *bet* gene products, and other features of Red-mediated recombination (STAHL, KOBAYASHI and STAHL 1985).

SZOSTAK and his collaborators performed a cross between a DNA segment with a double-stranded gap and a homologous DNA segment in yeast *Saccharomyces* around the same time (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; ORR-WEAVER and SZOSTAK

1983). They introduced into the yeast cells a plasmid with a double-stranded gap in a sequence homologous to a chromosomal sequence. They recovered products of which double-stranded gap had been repaired by copying of the homologous sequence. This gene conversion was sometimes accompanied by crossing over of the flanking sequences. Stimulated by these results, SZOSTAK *et al.* (1983) proposed that such double-stranded gap repair underlies meiotic homologous recombination in yeast. This model explains well the mating-type switching and the transposition of omega intron in mitochondria (STRATHERN *et al.* 1982; ZINN and BUTOW 1984) in yeast.

Similarity in the role of the double-strand breaks in *Escherichia coli* and in yeast led us to ask whether gene conversion takes place at a double-stranded gap in *E. coli.* We performed a cross between a gapped DNA segment and a homologous DNA segment in an *E. coli* strain and, indeed, recovered products that were predicted by the double-stranded gap repair model. In the accompanying paper (YAMAMOTO *et al.* 1988b), we analyze formation of the same gene conversion-type products without an artificial double-strand break.

MATERIALS AND METHODS

Bacterial strains and plasmids: JC8679 (GILLEN, WILLIS and CLARK 1974) is a generous gift from A. J. CLARK. The *recA1* strains, DH1 and DH5 (HANAHAN 1985) were described previously (YAMAMOTO *et al.* 1988a).

Construction of pIK43 is described elsewhere (YAMAMOTO *et al.* 1988a). Its repeats come from pSV2neo, which in turn is derived from Tn*5*. The left part is from pML2d, a derivative of pBR322. The right part comes from SV40 origin and from bovine papilloma virus type 1. The plasmid pIK42 is like pIK43 but without the *Xho*I linker.

Plasmid preparation: In order to avoid accumulation of the rare *neo*$^+$ recombinants, care was taken not to increase the generation number of a clone.

For small-scale analysis, one entire young colony was suspended in 5 ml of L broth with selective antibiotics (50 μg/ml ampicillin for the kanamycin-sensitive clones or 50 μg/ml kanamycin for all the kanamycin-resistant clones) and aerated at 37° to saturation. Plasmid DNA was prepared from 1–5 ml of the culture by the boiling method (HOLMES and QUIGLEY 1981).

For large scale preparation, the plasmid was introduced into a *recA1* strain (DH1 or DH5). The recombination frequency of DH1 [pIK43] to Neo$^+$ cells is as low as 4×10^{-7} per cell generation (YAMAMOTO *et al.* 1988a). A small scale culture (5 ml) from a single colony was used to check plasmid forms by the above method and then was used to start a larger culture (200 ml) with antibiotics. Plasmid DNA was prepared by a cleared lysate procedure in the presence of RNase after chloramphenicol amplification, concentrated with PEG6000, purified by CsCl/ethidium bromide centrifugation in the presence of Sarkosyl, and cleaned through phenol extraction, $CHCl_3$ extraction, and ethanol precipitation (YAMAMOTO *et al.* 1988a).

Transformation: The competent cells were prepared by the rubidium method as detailed by HANAHAN (1985). Aliquots of 0.20 ml of the competent cells in screw-capped micro tubes were instantly frozen and stored in liquid nitrogen. One tube of the competent cells was thawed at room temperature, placed on ice, mixed with DNA, which had been suspended in a small volume (less than 15 μl) of 10 mM Tris-HCl (pH 8) and 1 mM EDTA, and kept on ice for approximately 20 min. Immediately after 90 sec of heat shock at 42°, the tube was dipped in ice water. After 15 min, 1 ml of SOC broth (HANAHAN 1985) was added. The tube was incubated at 37° without shaking for 1 hr. The cells were spread on L agar plates with pH adjusted to 7.5 with Tris-HCl containing the kanamycin (50 μg/ml) or ampicillin (50–75 μg/ml) for overnight incubation at 37°. The transformation curves of JC8679 and DH5 using pBR322 are shown in Figure 2 below. The competent cells of JC8679 did not show saturation in the range examined in two preparations.

Analysis of the transformants: Analysis of the plasmids of the transformants by restriction enzyme digestion and gel electrophoresis was as described previously by YAMAMOTO *et al.* (1988a) and in a companion study (YAMAMOTO *et al.* 1988b).

RESULTS

Strategy. As a system to examine double-strand gap repair by gene conversion in *E. coli*, we chose, for convenience, plasmid recombination by a bacterial function homologous to lambda function instead of lambda recombination by lambda function. We tried intramolecular recombination as shown in Figure 1B. The plasmid has two homologous sequences in an inverted orientation, one of which is cuttable with a type II restriction enzyme to create a double-stranded gap. If gene conversion is intramolecular, it will be possible to recover both products of recombination on this plasmid even when the gene conversion event is accompanied by crossing over of the flanking sequences.

Figure 1A shows a map of the constructed plasmid, pIK43. This plasmid carries two copies of a sequence containing the *neo* (neomycin phosphotransferase) gene in an inverted orientation. The upper segment has a 283-bp deletion that removes one terminus of the *neo* gene. An 8-bp long *Xho*I linker sequence is inserted at this deletion. When this plasmid is cleaved with *Xho*I, the upper segment carries a long double-stranded gap compared with the lower segment.

We chose JC8679, an *E. coli* K-12 strain with *sbcA23 recB21 recC22* mutations (GILLEN, WILLIS and CLARK 1974) as the recipient bacteria that resemble lambda-infected cells. The *sbcA23* mutation activates the RecE pathway of homologous recombination which is homologous to the Red pathway of lambda and shows enhanced recombination near *cos* among unreplicated chromosomes (GILLEN, WILLIS and CLARK 1974). The *recB21* and *recC22* mutations inactivate exonuclease V (RecBCD enzyme), an enzyme that attacks double-stranded end (TAYLOR and SMITH 1985; OISHI, COSLOY and BASU 1974; SIMMON and LEDERBERG 1972). This enzyme interferes with lambda recombination enhancement near *cos* by the Red system (STAHL *et al.*

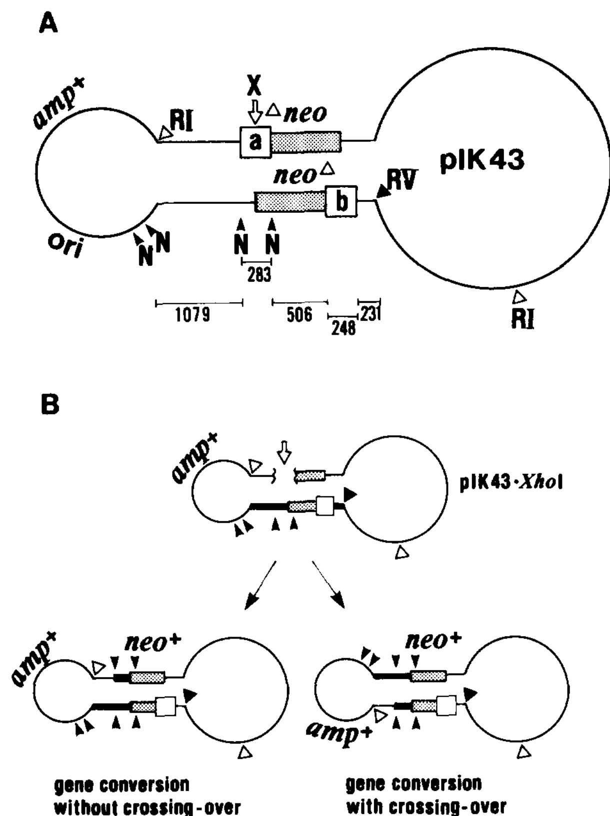

FIGURE 1.—(A) pIK43, the starting plasmid for the double-strand gap repair experiments. The two homologous duplex segments are drawn as parallel lines. The *neo* gene is from Tn*5* and confers kanamycin resistance to the host cell. The top segment has a deletion (deletion *a*) between the two *Nae*I sites, which removed one end (the C end) of the *neo* gene. The *Nae*I site was inactivated by insertion of an *Xho*I linker sequence, 5′ C-C-T-C-G-A-G-G. The bottom sequence has a 248-bp long deletion (deletion *b*), which removed the other end (the N end) of the *neo* gene. The left part (2321 bp), including the *amp* (ampicillin resistance) gene and the replication origin is derived from pBR322. The right part (8297 bp) is derived from SV40 and bovine papilloma virus type 1. The entire length is 14795 bp. The restriction enzymes and their site co-ordinates are: X: *Xho*I, 1082; N: *Nae*I, 11109, 11392, 12590, 12750; RI: *Eco*RI, 8024, 14793; RV: *Eco*RV, 10367. (B) Double-strand gap repair by intramolecular gene conversion. The double-stranded gap in the upper segment is repaired by copying the homologous sequence of the lower segment. This repair may take place with or without crossing over of the flanking sequences.

1974) and is inhibited by lambda's *gam* gene product in lambda infection (SAKAKI *et al.* 1973).

Detection of double-stranded gap repair by genetic selection: We cut the pIK43 plasmid with *Xho*I to make a double-stranded gap and introduced it into the competent cells of JC8679. The transformation curves of these competent cells obtained with pBR322 are shown in Figure 2.

We selected for kanamycin-resistant (Neo$^+$) colonies after transformation. This selection requires both restoration of a circular plasmid and reconstruction of the *neo*$^+$ gene by homologous recombination, and therefore it provides a direct selection for the double-stranded gap repair. Escape from digestion by restriction enzyme (*Xho*I) or simple rejoining of the ends will not result in a *neo*$^+$ plasmid upon transformation (see below). The number of the kanamycin-resistant transformants was increased about one order of magnitude by the *Xho*I treatment as shown in Table 1 and in Figure 2A. This increase was not observed by a double-stranded break with *Eco*RV at an irrelevant site (Figure 1A) as shown in Table 1.

All the examined transformants from pIK43/*Xho*I turned out to carry plasmids predicted by the double-strand break repair model as judged by analysis with restriction enzymes as summarized in Table 2. The plasmids retained their original size as judged by electrophoresis, and they gave a linear molecule of the original size upon *Eco*RV cut (Figure 3A). But the *Xho*I site that was present in the upper segment (Figure 1A) is gone. Instead, the two *Nae*I sites, which flank the deleted region and were absent in the upper segment (Figure 1A), have been regenerated in the upper segment of these *neo*$^+$ plasmids as seen in Figure 3B. This demonstrates repair of the double-stranded gap by gene conversion.

The restriction enzymes *Bgl*II (data not shown) and *Eco*RI (see below) gave patterns predicted by the double-strand gap repair model. Analysis with these enzymes revealed that 39% of these plasmids were indistinguishable from the starting plasmid, pIK43, except for the gene conversion at the gap (Table 2). They have the form shown in Figure 1A (bottom left).

Association of crossing over with gene conversion: Frequent association of crossing over is characteristic of gene conversion in eukaryotes (KITANI, OLIVE and EL-ANI 1962) and in *E. coli* (YAMAMOTO *et al.* 1988b). Many of our gene conversion products (61%) had undergone crossing over of the sequences flanking the conversion site as illustrated in Figure 1B (bottom right). Such crossing over changes the *Eco*RI pattern as shown in Figure 3C.

The control experiments show that the association of crossing over with gene conversion is not a result of two independent reactions. First, the pIK43 plasmid once established in JC8679 gives *neo*$^+$ plasmids of the crossing over type only at a low rate (YAMAMOTO *et al.* 1988b). Second, we prepared plasmid of the crossing-over type and plasmid of the non-crossing-over type in a *recA*$^-$ strain which allows very low recombination from pIK43 to *neo*$^+$ plasmids (YAMAMOTO *et al.* 1988b), and introduced each back into JC8679. We detected no signs of interconversion between the two types as detailed elsewhere (YAMAMOTO *et al.* 1988b). We concluded that the frequent association of crossing over we observed is characteristic of the gene conversion process repairing the double-stranded gap.

Evidence against alternative interpretations: Alternative explanations for the origin of the apparent

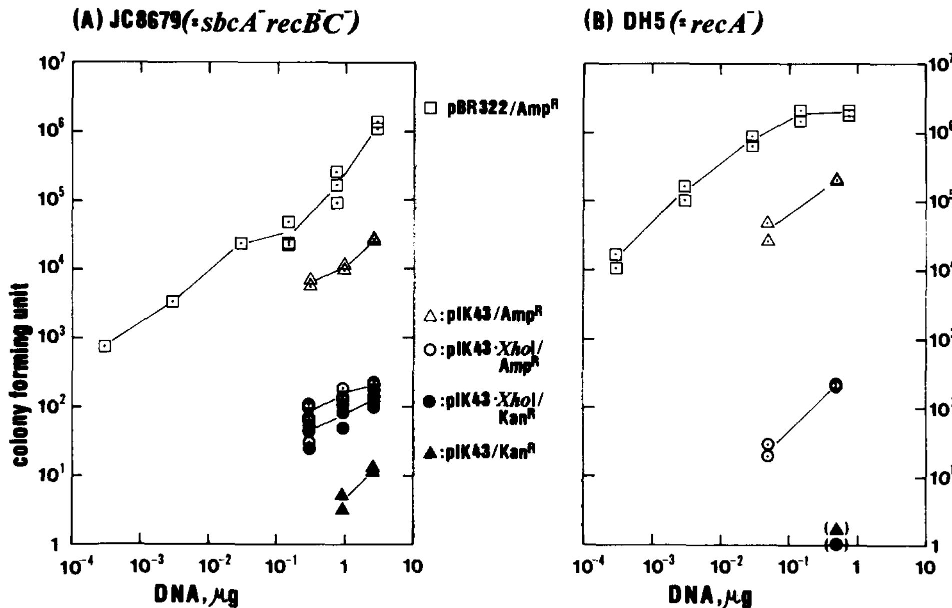

FIGURE 2.—Transformation at varying DNA inputs. A fixed amount of the competent cells (JC8679 or DH5) was incubated with varying amounts of plasmid DNA (intact pBR322, intact pIK43, or pIK43 cut with *Xho*I). The transformants (ampicillin-resistant or kanamycin-resistant) were selected for on agar. For transformation of JC8679 by pIK43/*Xho*I, fraction of 0.1 was plated on ampicillin agar and fraction of 0.9 was plated on kanamycin agar. Identical preparation of pIK43 was used in these experiments. (●) Less than one Kan^R colony in duplicate assays; (▲) less than one Amp^R colony in duplicate assays.

TABLE 1

Transformation by pIK43 with a double-stranded gap

Conditions		Colony-forming units	
Recipient	Enzyme	Kan^R	Amp^R
JC8679	None	1	5.5×10^3
		0	5.9×10^3
		3	6.6×10^3
		2	6.5×10^3
JC8679	*Xho*I	2.4×10^1	6×10^1
		3.3×10^1	1.4×10^2
		3.7×10^1	3×10^1
		3.5×10^1	1.1×10^2
JC8679	*Eco*RV	0	2.0×10^1
		0	2.5×10^1
		0	2.0×10^1
		0	1.5×10^1

pIK43 was cut with a large excess (30–100 times) of *Xho*I or *Eco*RV to completion as judged by ethidium visualization after gel electrophoresis. The reaction was stopped by adding EDTA to 20 mM and heating at 65° for 10 min. DNA was cleaned up by phenol/$CHCl_3$ extraction, ether or $CHCl_3$ extraction, and ethanol precipitation. Plasmid DNA (0.5 μg) in 10 mM Tris-HCl (pH 8) with 1 mM EDTA was incubated with the frozen competent cells prepared as described in MATERIALS AND METHODS. The fractions were spread on kanamycin agar plates or on ampicillin agar plates with pH adjusted to 7.5 with Tris-HCl buffer. The fractions of the culture plated on kanamycin agar were 0.98 for no enzyme, 0.9 for *Xho*I and 0.8 for *Eco*RV. The fractions plated on ampicillin agar were 0.02 for no enzyme, 0.1 for *Xho*I and 0.2 for *Eco*RV.

TABLE 2

Classification of the Kan^R product plasmids

	Conditions		Plasmids			
Experiment	Recipient	Enzyme	Total analyzed	Gene conversion without crossing over (%)	Gene conversion with crossing over (%)	Other (%)
1	JC8679	*Xho*I	6	67	33	0
2	JC8679	*Xho*I	48	35	65	0
1 + 2	JC8679	*Xho*I	54	39	61	0

The kanamycin-resistant colonies obtained by transformation of JC8679 with pIK43 cut with *Xho*I were grown in a small volume with kanamycin. Plasmid DNA was prepared and classified by size as determined by gel electrophoresis and by restriction patterns with *Nae*I, *Eco*RI, and *Bgl*II. Four clones in experiment 2 contained equal amounts of the two conversion types.

gene conversion products detected by kanamycin selection include: (1) intracellular formation from intact pIK43 which either escaped *Xho*I digestion or was reconstructed in the cell by end joining, (2) presence of the *neo*$^+$ plasmid in the starting plasmid preparation before transformation, and (3) bimolecular crossing over.

Possibility (1) (intracellular formation from intact pIK43) is excluded by the data in Table 1 and Figure 2A. Such escape or rejoining should give Amp^R transformants because pIK43 carries *amp*$^+$ gene. Decrease of Amp^R transformants of JC8679 by *Xho*I cut indicates such escape or rejoining is rare (approximately 10^{-2}). The *Xho*I treatment increased, rather than decreased, Kan^R transformants (by a factor of ten).

Possibility (2) (selection of *neo*$^+$ plasmids already present in the starting plasmid preparation) is excluded because of three lines of evidence. (*a*) The *Xho*I treatment increases Kan^R transformants as mentioned above. (*b*) Such *neo*$^+$ plasmids would give Neo$^+$ transformants even in a recombination-defective strain. No such Neo$^+$ transformant was detected in highly competent DH5 (*recA*$^-$) cells as shown in Figure 2B. (*c*) We cut pIK43 with *Xho*I and purified linear DNA through agarose gel electrophoresis off from uncut DNA that might have been present in the preparation. The purified DNA still produced Kan^R colonies when introduced into JC8679 as we describe elsewhere.

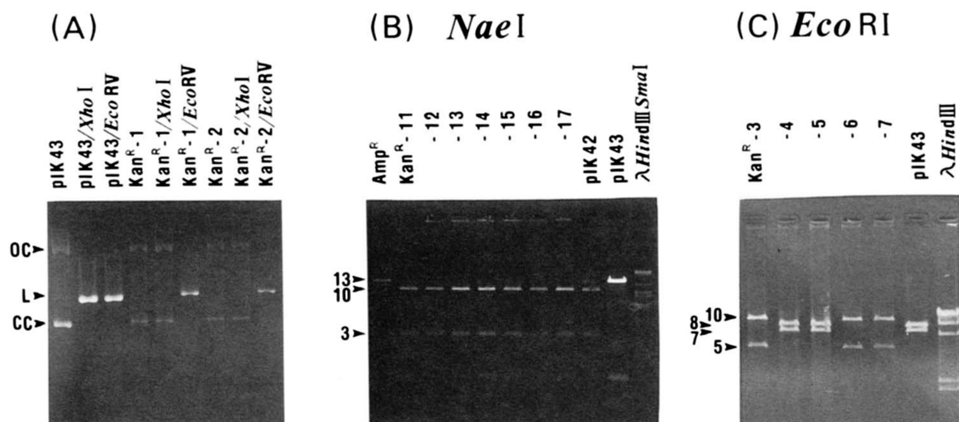

FIGURE 3.—Product plasmids analyzed with restriction enzymes. (A) No enzyme, *Xho*I and *Eco*RV to detect gene conversion. Kan^R – 1, Kan^R – 2, from the kanamycin-resistant transformants of JC8679 obtained with pIK43 cut with *Xho*I; OC, open circle; L, linear; CC, closed circle. (B) *Nae*I to detect gene conversion. Amp^R, from an ampicillin-resistant transformant of JC8679 obtained with intact pIK43; Kan^R, from the kanamycin-resistant transformants of JC8679 obtained with pIK43 cut with *Xho*I. The plasmids were transferred to DH1 (= *recA1*) for analysis. pIK42, like pIK43 but without the *Xho*I linker; 13, 10, 3, DNA fragment lengths in kilo base pairs. (C) *Eco*RI to detect crossing over. Kan^R, from the kanamycin-resistant transformants of JC8679 obtained with pIK43 cut with *Xho*I; 10, 8, 7, 5, DNA fragment lengths in kilobase pairs.

Possibility (3) (bimolecular crossing over) is also unlikely. This hypothesis must explain gene conversion type products with crossing over by two rounds of crossing over at two different sites between different pairs of DNA segments. One crossing over must take place to the left of the gap between the lower segment of the first plasmid and the upper segment of the second plasmid. Another crossing over must take place to the right of the gap between the upper segment of the first plasmid and the lower segment of the second plasmid.

In fact, when the amount of input DNA (pIK43/*Xho*I) was varied, the number of Kan^R colonies did not change as sharply as expected from second order kinetics as shown in Figure 2A. We believe that the estimate of the slope was not disturbed by saturation since the capacity of one tube of the competent cells of JC8679 had not reached saturation as assayed with intact pBR322 or intact pIK43 as seen in Figure 2A. We can not, however, exclude the possibility that circular and linear DNA show different saturation properties. For example the sticky ends might anneal and complicate this analysis.

Selection for Amp^R plasmids: The starting plasmid, pIK43, has an *amp* gene, which should be left intact after the *Xho*I digestion (Figure 1). In order to give an Amp^R colony, the plasmid has to become circular to be maintained in *E. coli*. This requires some kind of recombination. The selection of Amp^R colonies asks for establishment of circular plasmid with *amp*$^+$ gene and is less restrictive for the double-stranded gap repair.

We obtained Amp^R transformants of JC8679 by pIK43/*Xho*I as shown in Table 1. Most of the Amp^R plasmids belonged to the gene conversion types (Table 3). The apparent recombinant frequency was approximately 80%.

TABLE 3

Classification of the Amp^R product plasmids

	Conditions		Plasmids		
Experiment	Recipient	Enzyme	Total analyzed	Gene conversion (%)	Kan^S (%)
1	JC8679	*Xho*I	12	83[a]	17
	JC8679	*Eco*RV	6	0	100
	DH5	*Xho*I	6	0	100[b]
	DH5	*Eco*RV	6	0	100
2	JC8679	*Xho*I	12	83[c]	17
1 + 2	JC8679	*Xho*I	24	83	17

The ampicillin-resistant colonies obtained by transformation with pIK43 cut with *Xho*I or *Eco*RV were grown in a small volume with ampicillin selection. The diluted culture was spotted on a kanamycin agar plate and on an ampicillin agar plate. Clones that gave nearly the same plating efficiency on the two plates were classified as carrying a kanamycin-resistant plasmid. Plasmid DNA from the culture was run through 1% agarose gel before and after cleavage with *Nae*I and with *Eco*RI.

[a] Ten out of ten were gene conversion type with crossing over.

[b] One plasmid had a size larger than that of pIK43 monomer.

[c] Nine were gene conversion type with crossing over, and one was gene conversion type without crossing over.

Four out of 24 of the Amp^R plasmids in JC8679 from pIK43/*Xho*I were Kan^S (Table 3). These were analyzed with *Xho*I, *Nae*I, *Eco*RI and *Bgl*II. Three were found to be indistinguishable from the starting plasmid pIK43. They are likely to have resulted from intracellular rejoining of the cut ends during transformation or from escape from the *Xho*I digestion before transformation.

One Amp^R plasmid in JC8679 from pIK43/*Xho*I has apparently resulted from nonhomologous rearrangements (Table 3, Experiment 2). We do not know whether it was present in the starting plasmid preparation for transformation or formed in the cell during transformation. We have not characterized further this plasmid.

The Amp^R transformants obtained with pIK43 cut at an irrelevant site with *Eco*RV (see Figure 1A) was less than those with pIK43/*Xho*I (Table 1). This is consistent with contribution of the double-strand gap repair with pIK43/*Xho*I. None of these Amp^R transformants was Kan^R (Table 3) as expected.

The Amp^R transformants by pIK43/*Xho*I were less in the recombination-deficient strain DH5 than in JC8679 as seen in Figure 2: The ratio of [Amp^R from pIK43/*Xho*I]/[Amp^R from pIK43] was approximately 10^{-2} in JC8679 but less than 10^{-3} in DH5. This is again consistent with contribution of the double-strand gap repair in JC8679. None of these Amp^R transformants of DH5 from pIK43/*Xho*I was Kan^R (Table 3) as expected.

DISCUSSION

By introducing a plasmid molecule with a large (300 bp) double-stranded gap into in *E. coli* strain we recovered products in which the gap had been repaired apparently by gene conversion from a homologous sequence on the same molecule. Gene conversion was frequently accompanied by crossing over of the flanking sequences as in eukaryotes (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981; JAYARAM 1986). These results provide support to the double-strand break repair model of homologous recombination (SZOSTAK *et al.* 1983) in a prokaryotic organism, *E. coli.*

Increase of exchange in the RecE pathway by double-strand breaks was observed in experiments in which only one product of recombination was recovered (SYMINGTON, MORRISON and KOLODNER 1985). The enhancement of homologous recombination in lambda's own Red pathway at *cos* by terminase (see Introduction) or at *Eco*RI sites by *Eco*RI endonuclease (THALER, STAHL and STAHL 1987a,b) has been explained well by the double-stranded gap repair mechanism. Alternatively, the double-strand break might promote these exchanges in a physically nonreciprocal way. Physical non-reciprocality was observed at *cos* in a $recA^-$ red^+ case (STAHL and STAHL 1985). The double HOLLIDAY structure postulated in the double-strand break repair model was detected in λ in an *E. coli* strain with a different genetic background (KOBAYASHI and IKEDA 1983).

How efficient is our double-strand gap repair? The number of Kan^R transformants increased ten fold by the cut (Table 1 and Figure 2A). We conclude that the recombination enhancement by the double-strand gap is quite efficient. This increase is larger or, at least, comparable to the increase (fivefold) by a double-strand break in an experiment in which recovery of only one product, instead of two, was required and repair of a long (283 bp) gap was not required (SYMINGTON, MORRISON and KOLODNER 1985). The yield of Amp^R transformant was decreased 100 fold by the cut (Table 1 and Figure 2A). This number does not impress as indicating efficient repair at the first glance. But the ratio [number of Amp^R from cut DNA]/[number of Amp^R from intact DNA] is less than 1/1000 in a recombination-deficient strain (Figure 2B and last section of RESULTS). This difference reflects action of efficient repair in JC8679. Our experimental design (Figure 1B) was such that the only routes (by homologous recombination) from cut plasmid to Amp^R transformants (physically recombinant) are those yielding Kan^R plasmids (genetically recombinant). We found indeed that most of the Amp^R plasmids from cut plasmids are Kan^R (Table 3 and last section of RESULTS). The apparent "recombinant frequency" ([number of Kan^R Amp^R]/[number of Amp^R]) was as high as 80% for cut plasmid. This exclusion of silent recombinants is one difference from usual recombination experiments.

The double-strand breaks enhance homologous recombination in a different way in the RecBCD pathway. The RecBCD enzyme enters the DNA at a double-stranded end and travels along the DNA until it encounters a special site, called chi, and stimulates exchange in a physically reciprocal fashion (KOBAYASHI *et al.* 1982, 1984; KOBAYASHI, STAHL and STAHL 1984; STAHL *et al.* 1983, 1986).

These mechanisms of homologous recombination may contribute to the repair of the double-strand breaks on the chromosome (TOMIZAWA and OGAWA 1968; KRASIN and HUTCHINSON 1977). The double-stranded end either present in DNA introduced into the cell in conjugation or in general transduction or made during rolling-circle DNA replication may stimulate homologous recombination through one of these mechanisms.

In a companion study (YAMAMOTO *et al.* 1988b), we analyze formation of the same gene conversion-type products from the same plasmid in the absence of an artificial double-stranded gap.

We would like to thank A. J. CLARK for his generous gift of bacterial strains. We are grateful to K. YAMAMOTO, H. YOSHIKURA, Y. KITAMURA and A. NOMOTO for their discussions. We also would like to thank F. W. STAHL, M. S. FOX, and H. IKEDA for motivating us. We appreciate critical reading of the manuscripts by M. OISHI. We appreciate help in the course of publication by N. KLECKNER and J. W. DRAKE. An earlier version was processed in a computer by Y. KAYANOKI, T. MUTO, and Y. MIYAMOTO. This work was supported by grants to I. KOBAYASHI from the Japanese Ministry of Education, the Japanese Ministry of Health, and Mochida Pharmaceutical Foundation (Tokyo) and also, at an early stage, by grants to H. YOSHIKURA and to A. NOMOTO.

LITERATURE CITED

GILLEN, J. R., D. K. WILLIS and A. J. CLARK, 1974 The recE pathway of bacterial recombination. pp. 123–135. In: *Mechanisms in Genetic Recombination*, Edited by R. F. GRELL. Plenum, New York.

HANAHAN, D., 1985 Techniques for transformation of *E. coli.* pp.

109–135. In: *DNA Cloning, Vol. I*, Edited by D. M. GLOVER. IRL Press, Oxford.

HOLMES, D. S., and M. QUIGLEY, 1981 A rapid boiling method for the preparation of bacterial plasmids. *Anal. Biochem.* **114:** 193–198.

JAYARAM, M., 1986 Association of reciprocal exchange with gene conversion between the repeated segments of 2-micrometer circle. *J. Mol. Biol.* **191:** 341–354.

KITANI, Y., L. S. OLIVE and A. S. EL-ANI, 1962 Genetics of *Sordaria fimicola*. V. Aberrant segregation at the *g* locus. *Am. J. Bot.* **49:** 697–706.

KMIEC, E., and W. K. HOLLOMAN, 1981 β Protein of bacteriophage promotes renaturation of DNA. *J. Biol. Chem.* **256:** 12636–12639.

KOBAYASHI, I., and H. IKEDA, 1983 Double Holliday structure: a possible in vivo intermediate form of general recombination in *Escherichia coli. Mol. Gen. Genet.* **191:** 213–220.

KOBAYASHI, M. M. STAHL and F. W. STAHL, 1984 The mechanism of the chi-*cos* interaction in RecA-RecBC-mediated recombination in phage lambda. *Cold Spring Harbor Symp. Quant. Biol.* **49:** 497–506.

KOBAYASHI, I., H. MURIALDO, J. M. CRASEMAN, M. M. STAHL and F. W. STAHL, 1982 Orientation of cohesive end site cos determines the active orientation of chi sequence in stimulating *recA recBC* mediated recombination in phage lambda lytic infections. *Proc. Natl. Acad. Sci. USA* **79:** 5981–5985.

KOBAYASHI, I., M. M. STAHL, D. LEACH and F. W. STAHL, 1983 The interaction of *cos* with Chi is separable from DNA packaging in *recA-recB*-mediated recombination of bacteriophage lambda. *Genetics* **104:** 549–570.

KOBAYASHI, I., M. M. STAHL, F. R. FAIRFIELD and F. W. STAHL, 1984 Coupling with packaging explains apparent nonreciprocality of Chi-stimulated recombination of bacteriophage lambda by RecA and RecBC functions. *Genetics* **108:** 773–794.

KRASIN, F., and F. HUTCHINSON, 1977 Repair of DNA double-strand breaks in *Escherichia coli*, which requires *recA* function and the presence of a duplicate genome. *J. Mol. Biol.* **116:** 81–98.

MUNIYAPPA, K. and C. M. RADDING, 1986 The homologous recombination system of phage λ: Pairing activities of β protein. *J. Biol. Chem.* **261:** 7472–7478.

OISHI, M., S. D. COSLOY and S. K. BASU, 1974 Transformation-mediated recombination in *Escherichia coli*. pp. 145–154. In: *Mechanisms in Genetic Recombination*, Edited by R. F. GRELL. Plenum, New York.

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

ORR-WEAVER, T. L., J. W. SZOSTAK and R. J. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. *Proc. Natl. Acad. Sci. USA* **79:** 6354–6358.

SAKAKI, Y., A. E. KARU, S. LINN and H. ECHOLS, 1973 Purification and properties of the gamma-protein specified by phage lambda: an inhibitor of the host recBC recombination enzyme. *Proc. Natl. Acad. Sci. USA* **70:** 2215–2219.

SIMMON, V. F., and S. LEDERBERG, 1972 Degradation of bacteriophage lambda deoxyribonucleic acid after restriction by *Escherichia coli* K-12. *J. Bacteriol.* **112:** 161–168.

SMITH, G. R., 1983 General recombination, in Lambda II. pp. 175–209. In: *Lambda II*, Edited by R. HENDRIX, J. ROBERTS, F. STAHL and R. WEISBERG. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

STAHL, F. W., 1979a *Genetic Recombination*. Freeman, San Francisco.

STAHL, F. W., 1979b Special sites in generalized recombination. *Annu. Rev. Genet.* **13:** 7–24.

STAHL, F. W., 1986 Roles of double-strand breaks in generalized genetic recombination. *Progr. Nucleic Acid Res. Mol. Biol.* **33:** 169–194.

STAHL, F. W., and M. M. STAHL, 1985 Non-reciprocal crossing-over in phage lambda. *J. Genet.* **64:** 31–39.

STAHL, F. W., I. KOBAYASHI and M. M. STAHL, 1982 Distance from cohesive end site, *cos*, determines the replication requirement for recombination in phage lambda. *Proc. Natl. Acad. Sci. USA* **79:** 6318–6321.

STAHL, F. W., I. KOBAYASHI and M. M. STAHL, 1985 In phage lambda, *cos* is a recombinator for Red pathway. *J. Mol. Biol.* **181:** 199–209.

STAHL, F. W., K. D. MCMILIN, M. M. STAHL, J. M. CRASEMANN and S. LAM, 1974 The distribution of crossovers along unreplicated lambda phage chromosomes. *Genetics* **77:** 395–408.

STAHL, M. M., I. KOBAYASHI, F. W. STAHL and S. K. HUNTINGTON, 1983 Activation of chi, a recombinator, by the action of endonuclease at a distant site. *Proc. Natl. Acad. Sci. USA* **80:** 2310–2313.

STAHL, F. W., I. KOBAYASHI, D. THALER and M. M. STAHL, 1986 Direction of travel of recBC recombinase through bacteriophage lambda DNA. *Genetics* **113:** 215–227.

STRATHERN, J. N., A. J. S. KLAR, J. B. HICKS, J. A. ABRAHAM, J. M. IVY, K. A. NASMYTH and C. MCGILL, 1982 Homothallic switching of yeast mating type cassettes is initiated by a double-strand cut in the MAT locus. *Cell* **31:** 183–192.

SYMINGTON, L. S., P. MORRISON and R. KOLODNER, 1985 Intramolecular recombination of linear DNA catalyzed by the *Escherichia coli* RecE recombination system. *J. Mol. Biol.* **186:** 515–525.

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:** 25–33.

TAYLOR, A. F., and G. R. SMITH, 1985 Substrate specificity of the DNA unwinding activity of the RecBC enzyme of *Escherichia coli. J. Mol. Biol.* **185:** 431–443.

THALER, D. S., M. M. STAHL and F. W. STAHL, 1987a Double-chain-cut-sites are recombination hot spots in the Red pathway of phage lambda. *J. Mol. Biol.* **195:** 75–87.

THALER, D. S., M. M. STAHL and F. W. STAHL, 1987b Tests of the double-strand-break repair model for Red-mediated recombination of phage λ and plasmid λ-dv. *Genetics* **116:** 501–511.

TOMIZAWA, J., and H. OGAWA, 1968 Breakage of DNA in *rec*$^+$ and *rec*$^-$ bacteria by disintegration of radiophosphorus atoms in DNA and possible cause of pleiotropic effects of *recA* mutation. *Cold Spring Harbor Symp. Quant. Biol.* **33:** 243–251.

WHITE, R. L., and M. S. FOX, 1974 On the molecular basis of high negative interference. *Proc. Natl. Acad. Sci. USA* **71:** 1544–1548.

YAMAMOTO, K., H. YOSHIKURA, N. TAKAHASHI and I. KOBAYASHI, 1988a Apparent gene conversion in *Escherichia coli rec*$^+$ strain is explained by multiple rounds of reciprocal crossing-over. *Mol. Gen. Genet.* **213:** 393–404.

YAMAMOTO, K., N. TAKAHASHI, H. YOSHIKURA and I. KOBAYASHI, 1988b Homologous recombination involving a large heterology in *Escherichia coli*. *Genetics* **119:** 759–769.

ZINN, A. R., and R. A. BUTOW, 1984 Non-reciprocal exchange between alleles of the yeast mitochondrial 21S rRNA gene: kinetics and the involvement of a double-strand break. *Cell* **40:** 887–895.

Communicating editor: F. W. STAHL