Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1762–1766, March 1986
Genetics

Double-strand gap repair results in homologous recombination in mouse L cells

(gene transfer/thymidine kinase gene/gene conversion/mammalian recombination/nonreciprocal recombination)

DAVID A. BRENNER†, ANN C. SMIGOCKI‡, AND R. DANIEL CAMERINI-OTERO

Genetics and Biochemistry Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205

Communicated by J. E. Rall, October 17, 1985

ABSTRACT **Previous studies have demonstrated that the presence of double-strand breaks or double-strand gaps increases the frequency of homologous recombination between two cotransferred DNAs when they are introduced into cultured mammalian cells. Here we demonstrate that the repair of these double-strand gaps is a major mechanism for homologous recombination between exogenous DNAs. In particular, when a plasmid DNA containing a 104-base-pair (bp) gap in its *tk* gene (herpes simplex virus gene for thymidine kinase) undergoes recombination in mouse L cells to generate an intact gene, the majority of events result from direct repair of the double-strand gap using a cotransferred DNA as the template. We analyzed the recombination events by comparing the frequency of tk^+ colonies, Southern blotting of cloned tk^+ cell lines, and cloning recombined functional *tk* genes by plasmid rescue. In addition, by creating double-strand breaks within or adjacent to heterologous insertions in a mutant *tk* gene, we estimate that the L cell can generate a double-strand gap of between 152 and 248 bp and then can repair the gap to create a functional *tk* gene. We conclude that double-strand breaks and double-strand gaps are recombinogenic in transferred plasmid DNAs because they serve as intermediates in homologous recombination by double-strand gap repair, a nonreciprocal exchange of DNA or gene conversion event.**

The homologous recombination between exogenous DNAs introduced into cultured mammalian somatic cells is a very efficient process. The technique by which the DNAs are introduced into the cell does not appear to be critical, since homologous recombination between transferred DNAs has been studied by using calcium phosphate-mediated gene transfer (1–10), microinjection (11, 12), and protoplast fusion (13). However, altering the DNA substrates prior to their cotransfer into cells does affect the frequency of recombination. We (6, 10) and others (7–9) have demonstrated that the creation of a double-strand break or a double-strand gap in a DNA prior to cotransfer with an uncut plasmid increases the frequency of homologous recombination between the two DNAs compared to the recombination frequency of two uncut plasmids.

When DNAs containing double-strand gaps up to 1200 base pairs (bp) in size are introduced into mitotic yeast cells, they are efficiently repaired by the nonreciprocal exchange of genetic information, a gene conversion event (14, 15). This finding led to a model (16) in which double-strand breaks are enlarged into double-strand gaps and then repaired by DNA synthesis with homologous DNA as a template.

In the present study, we demonstrate that double-strand gap repair is a mechanism for the homologous recombination between pairs of cotransferred plasmids containing mutations in the herpes simplex virus (HSV) gene *tk* encoding thymidine kinase in mouse LTK^- cells. The plasmids containing the mutant *tk* genes contain diagnostic restriction sites that allowed us to study the products of recombination by using Southern blots and plasmid rescues. We demonstrate that a 104-bp gap in *tk* is accurately repaired to regenerate a functionally active *tk* gene. We also show that a double-strand break in a mutant *tk* gene can be expanded to a double-strand gap of approximately 152–248 bp and then repaired to generate a functional *tk* gene.

MATERIALS AND METHODS

Plasmid Constructions. Plasmid pTK was constructed by inserting the 2.0-kilobase-pair (kbp) *Pvu* II fragment (17) containing the functional *tk* gene into the *Sma* I site of pUC8 oriented in such a manner that the distance from the *Bam*HI cloning site to the *Nru* I site of the *tk* gene is 600 bp. Plasmid pBS (Fig. 1*A*), which contains the 5′ end of the *tk* gene (the 1.2-kbp *Bam*HI–*Sph* I fragment) was constructed as described (10).

The pSTI family of plasmids contain heterologous insertions into the *Nru* I site of the coding region of *tk* in pTK, where I is the size of the heterologous insertion in bp (10). The insert in pST611 is oriented in such a manner that the distance from the *Bam*HI cloning site to the *Hpa* I site in the insert is 720 bp. Plasmid pERV8 was constructed by deleting the 104-bp *Eco*RV fragment in the coding region of *tk* in pTK as described (10). The 2.1-kbp *Bam*HI–*Pvu* II fragment containing the deleted *tk* gene of pERV8 was ligated into the *Bam*HI and *Sma* I sites of pUC12 to generate pERV12 and of pUC13 to generate pERV13. The pERV family of plasmids consists of pERV8, pERV12, and pERV13 (Fig. 1*B*). The preparations, concentration determinations, and restriction endonuclease digestions of the plasmids were as described (10).

DNA-Mediated Gene Transfer and Southern Blotting. DNA-mediated gene transfer into the recipient LTK^- cell line was performed by using the calcium phosphate precipitation technique (18) as described (19, 20). A 4-μg sample of each mutant *tk* plasmid plus 20 μg of LTK^- high molecular weight carrier DNA was added to 10^6 LTK^- cells. The cloning of the resulting tk^+ cell lines, the preparation of high molecular weight DNA, and the Southern blotting (21) were performed as described (10, 19).

Plasmid Rescue. Cellular DNA (100 μg) from transformed cloned tk^+ cell lines was digested with *Bam*HI. The protocol for plasmid rescue was as described (19, 22) and also by a modified plasmid rescue technique using the lac repressor protein (A.S., unpublished data). We examined a total of 107

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: bp, base pair(s); HSV, herpes simplex virus.

†Present address: Division of Gastroenterology, University of California at San Diego Medical Center, 225 Dickinson Street, San Diego, CA 92103.
‡Present address: Tissue Culture and Molecular Genetics Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD 20705.

rescued plasmids derived from five different cloned cell lines. Thirty-two of these rescued plasmids have intact *tk* genes as a result of recombination events between parental plasmids.

RESULTS

Double-Strand Gaps and Double-Strand Breaks in Deletion Mutants. The results of cotransferring the plasmids pBS and pERV8 into LTK$^-$ cells demonstrate the recombinogenic effect of creating a double-strand gap or a double-strand break in one of the pair of substrate molecules. The plasmid pERV8 contains the entire *tk* gene minus a 104-bp deletion in the coding region that removes the unique *Nru* I site. It was constructed by deleting the 104-bp *Eco*RV fragment in the coding region of *tk* (Fig. 1*D*). The plasmid pBS contains the 5′ end of *tk* including the DNA deleted from pERV8 plus the additional 55 bp of homologous DNA lying between the *Eco*RI and *Sph* I sites (Fig. 1*D*). When any of our plasmids containing mutant *tk* genes are transferred individually into LTK$^-$ cells, they never produce any *tk*$^+$ colonies.

When uncut pBS and uncut pERV8 are cotransferred into LTK$^-$ cells, they produce an average of 5.5 *tk*$^+$ colonies (ref. 10; Table 1). When pERV8 is cut with the restriction endonuclease *Eco*RV, a 104-bp double-strand gap is created in comparison with an intact *tk* gene. When uncut pBS and *Eco*RV-cut pERV8 are cotransferred, the number of *tk*$^+$ colonies increases to 104. When pERV8 is cut with the restriction endonuclease *Bgl* II to create a double-strand break, the number increases to 58 *tk*$^+$ colonies (ref. 10; Table 1).

The mutant *tk* gene with the 104-bp deletion was cloned into three different vectors (pUC8, pUC12, and pUC13) to study the effect of the orientation of *tk* within the vector and to obtain additional unique restriction endonuclease sites flanking the *tk* gene. These additional experiments demonstrated that the orientation of *tk* did not affect the frequency of recombination (Table 1). The average effect of creating a double-strand gap was a 13-fold increase in the recombination frequency compared to the uncut plasmid. The average effect of creating a double-strand break was a 9-fold increase in the recombination frequency.

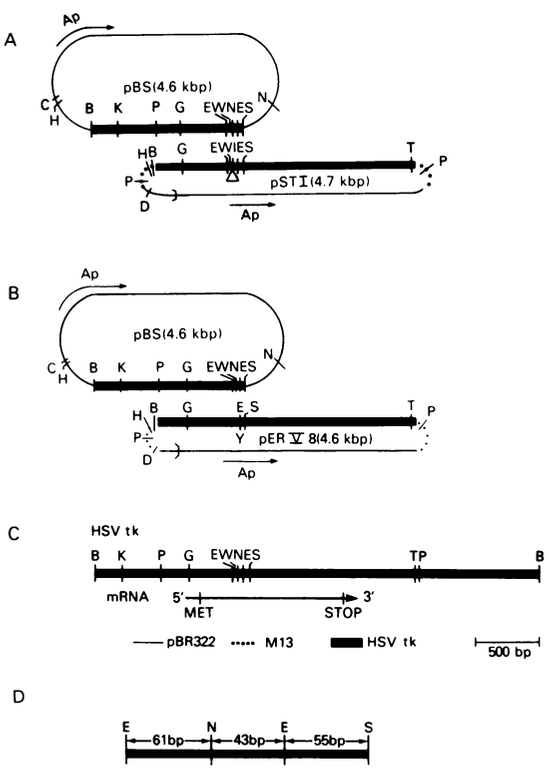

FIG. 1. (*A* and *B*) Restriction endonuclease maps of plasmids constructed for homologous recombination experiments. (*C*) Original 3.5-kbp HSV type 1 CL101 *Bam*HI fragment O containing *tk*. A, *Sac* I; B, *Bam*HI; C, *Cla* I; D, *Nde* I; E, *Eco*RV; G, *Bgl* II; H, *Hin*dIII; K, *Kpn* I; N, *Nru* I; P, *Pvu* II; S, *Sph* I; T, *Bst*EII; W, *Sna*BI; I, site of insertion of heterologous DNA; Y, site of 104-bp deletion. (*D*) Enlargement of the segment of *tk*.

Table 1. Numbers of *tk*$^+$ colonies resulting from the cotransfer of pairs of different mutant plasmids

Type of cotransferred plasmids	Specific second plasmid	Relative no. of *tk*$^+$ colonies per plate*
pBS × uncut pERV	pERV8	5.5 (1–8)
	pERV12	10 (8–12)
	pERV13	8.5 (7–10)
pBS × *Eco*RV-cut pERV	pERV8	104 (44–148)
	pERV12	94 (88–100)
	pERV13	104 (99–109)
pBS × *Bgl* II-cut pERV	pERV8	58 (18–90)
	pERV12	80 (58–103)
	pERV13	88 (85–91)
pBS × insertion-cut pSTI	*Stu* I-cut pST8	183 (86–375)
	Xba I-cut pST24	167 (160–175)
	Hpa I-cut pST611	8.5 (5–12)
pBS × *Sna*BI-cut pSTI	pST8	231 (182–280)
	pST20	290 (238–341)
	pST24	284 (266–303)
	pST72	77 (54–89)
	pST126	45 (37–53)
	pST318	52 (37–67)

*tk$^+$ colonies were counted after 14 days in hypoxanthine/aminopterin/thymidine selective medium. The number of *tk*$^+$ colonies per plate was normalized to the number of *tk*$^+$ colonies in the pBS × pST8 plate in each experiment. The numbers in parentheses show the range over multiple experiments.

There are three possible mechanisms by which an intact *tk* gene can be generated by the recombination between uncut pBS and double-strand gapped pERV (Fig. 2): recombination without gap repair, gap repair without exchange of flanking markers, and gap repair with exchange of flanking markers (16). We can use *B-N-X* [where *B* is the *Bam*HI site located 1100 bp from the *Nru* I site (*N*) and *X* is any site to the right of *N*] to denote the genotype of pBS and *B*-gap-T* (where B* is the *Bam*HI site in pERV and *T* is its *Bst*EII site) to denote the genotype of gapped pERV. If *B-N-X* is crossed with *B**-gap-*T*, gap repair without exchange of flanking markers will result in an intact *tk* gene with the *B*-N-T* genotype. The other mechanisms that lead to a wild-type *tk* gene will

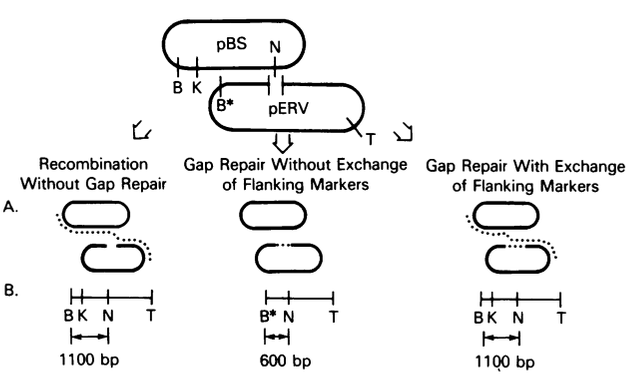

FIG. 2. (*A*) Three possible mechanisms by which uncut plasmid pBS and plasmid pERV with a double-strand gap can recombine to generate an intact *tk* gene. (*B*) Simplified restriction endonuclease map of intact *tk* generated by recombination. The predicted length of the *Bam*HI–*Nru* I restriction fragment is shown. Abbreviations are as in Fig. 1, except for B* (*Bam*HI) in pERV (see text).

generate products with *B-N-T* genotypes. Experimentally, both genotypes can be distinguished by the difference in restriction fragment lengths between *B-N* (1100 bp) and *B*-N* (600 bp). A 600-bp fragment is characteristic of gap repair without exchange of flanking markers. An 1100-bp fragment is characteristic of a recombination product with the 5′ end of *tk* coming from the parental pBS plasmid.

Southern blots of cloned tk^+ cell lines resulting from the cotransfer experiments are shown in Fig. 3*A* (*Eco*RV-cut pERV × pBS) and Fig. 3*B* (*Bgl* II-cut pERV × pBS). The cellular DNAs were digested with *Bam*HI and *Nru* I, and the filters were probed with a 3.5-kbp fragment containing *tk*. In addition, the plasmid pTK with an intact *tk* cloned into pUC8 was digested with *Bam*HI and *Nru* I to generate an identical 600-bp fragment to serve as a reference for the lanes containing digests of cellular DNA. In five of six tk^+ lines resulting from the cotransfers with *Eco*RV-cut pERV containing a double-strand gap, the characteristic 600-bp fragment was present (Fig. 3*A*, lanes 2, 4, 5, 6, and 7). In contrast, only the DNA from one of five of the tk^+ lines resulting from the cotransfers with *Bgl* II-cut pERV containing a double-strand break 347 bp to the left of the deletion in *tk* showed a characteristic 600-bp band (Fig. 3*B*, lane 4). The 600-bp band in the Southern blot from cell line 189 (Fig. 3*B*, lane 4) was faint but clearly visible in the original autoradiogram.

We cloned the intact *tk* genes responsible for the thymidine kinase-positive phenotype in several cell lines by the technique of plasmid rescue (Fig. 4 *A* and *B*); we shall refer to plasmids responsible for the thymidine kinase-positive phenotype as tk^+ plasmids. Our purpose was to clarify the results of the Southern blots in order to determine whether the new 600-bp *Bam*HI–*Nru* I restriction fragment was actually from an intact *tk* gene and not from a reciprocal recombinant lacking a functional *tk* gene (see Fig. 2). We rescued plasmids from three independent cloned cell lines (186, 208, and 219) resulting from the cotransfer of pBS and *Eco*RV-cut pERV, all of which demonstrated the 600-bp *Bam*HI–*Nru* I restriction fragment on Southern blots (Fig. 3*A*). There were two different tk^+ rescued plasmids from lines 186 and 219 and one tk^+ rescued plasmid from line 208 (Fig. 4*A*). All five of the tk^+ rescued plasmids have the characteristic 600-bp *Bam*HI–*Nru* I restriction fragment. Therefore, all five appeared to result from gap repair without exchange of flanking markers. In addition, the rescued plasmids derived from the cotransfer of pERV13 (p208B4, p219B2, and p219B23) contained the expected unique restriction enzyme sites *Xba* I (X) and *Sac* I (A). Besides containing the result of a gap repair event, two of the tk^+ rescued plasmids (p186B3 and p219B2) contain DNA sequences resulting from additional recombination events.

We rescued one tk^+ plasmid from the cell line 199, which does not contain the 600-bp *Bam*HI–*Nru* I restriction fragment on the Southern blot (Fig. 3*A*). The tk^+ rescued plasmid, p199B, which conferred the thymidine kinase-positive phenotype, contained an 1100-bp *Bam*HI–*Nru* I restriction fragment characteristic of recombination without gap repair or gap repair with exchange of flanking markers (Fig. 4*A*).

The cell line 189 was the only cell line from the *Bgl* II-cut pERV experiments that contained a 600-bp restriction fragment on the Southern blot (Fig. 3*B*, lane 4). The tk^+ plasmid p189B15 rescued from cell line 189 contains the 1100-bp *Bam*HI–*Nru* I restriction fragment as well as other unique restriction sites (such as *Kpn* I); this arrangement is consistent with the plasmid pBS providing the 5′ end of the *tk* gene (Fig. 4*B*). Another tk^+ plasmid rescued from cell line 189, p189B28, has a short fragment at the 5′ end of the *tk* gene that was probably not derived from either pBS or pERV since it lacked the expected *Bgl* II restriction site (Fig. 4*B*). The 600-bp band detected in the Southern blot of the DNA of cell line 189 may represent the product of a recombination event between the 5′ end of pERV and the 3′ end of pBS. Therefore, none of the functional *tk* genes resulting from the cotransfer of pBS and *Bgl* II-cut pERV were the product of a simple gap repair of the 104-bp deletion.

Double-Strand Breaks Within Heterologous Insertions. If gap repair is an important mechanism in a eukaryotic cell, we would expect the cell to generate gaps at double-strand breaks and use a homologous DNA template to repair the gap. We used a previously described family of plasmids, each with insertions of heterologous DNA into the unique *Nru* I site of *tk*, called the pSTI plasmids (ref. 10; Fig. 1*A*) to demonstrate that double-strand gaps can accommodate insertions of 24 bp but not 611 bp in the recombination process.

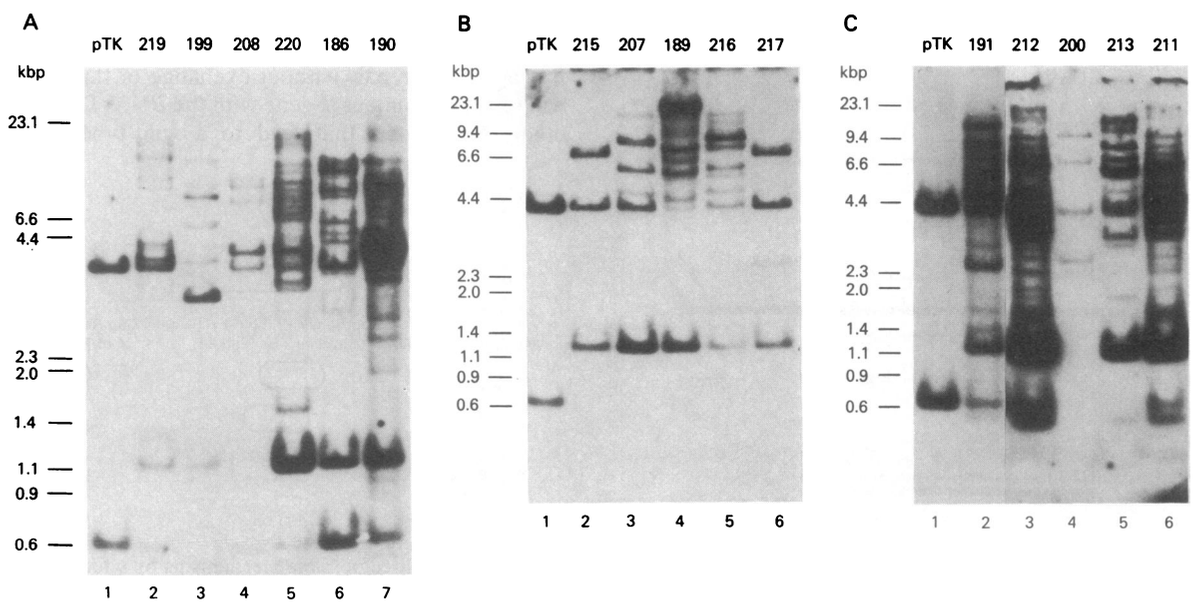

FIG. 3. Southern blots of plasmid pTK DNA and cellular DNA from cloned tk^+ cell lines and probed with radioactively labeled *tk*. The tk^+ cell lines were derived from the cotransfer of the following pairs of plasmids into mouse LTK^- cells. (*A*) *Eco*RV-cut pERV and uncut pBS. (*B*) *Bgl* II-cut pERV and uncut pBS. (*C*) *Stu* I-cut pST8 and uncut pBS.

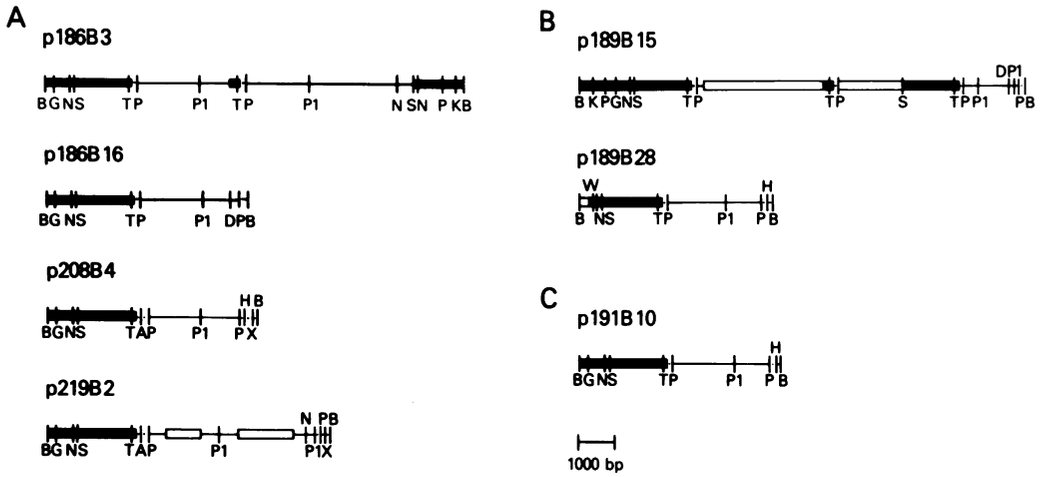

FIG. 4. Restriction endonuclease maps of plasmids rescued from cloned tk^+ cell lines. The numbers following the letter p refer to the cell line from which the plasmid was rescued. The open box designates unknown DNA. P1, *Pvu* I; other abbreviations are as in Fig. 1. (*A*) *Eco*RV-cut pERV × pBS. (*B*) *Bgl* II-cut pERV × pBS. (*C*) *Stu* I-cut pST8 × pBS.

We reasoned that if the cell could extend a double-strand break in pSTI into a gap of sufficient size to encompass a heterologous insertion, then the gap could subsequently be repaired by using pBS as a template. In such a circumstance, we would expect the cotransfer of pBS with cut pSTI to have a higher frequency of recombination when compared to the cotransfer of pBS with uncut pSTI. When pST8 with an 8-bp insertion is cut at its unique *Stu* I site within the insert, then the recombination frequency with cotransferred pBS is increased 3.5-fold compared to uncut pST8 (Tables 1 and 2). When pST24 with a 24-bp insertion is cut at its unique *Xba* I site centered within the insert, then the recombination frequency with cotransferred pBS is increased 4.3-fold. However, cutting pST611 with a 611-bp insertion at its unique *Hpa* I site did not increase the recombination frequency (Tables 1 and 2).

Three of the five cloned tk^+ cell lines resulting from the cotransfer of pBS with *Stu* I-cut pST8 have the 600-bp *Bam*HI–*Nru* I restriction fragment characteristic of gap repair without exchange of flanking markers (Fig. 3*C*). Restriction mapping of the tk^+ plasmids rescued from one of these cell lines (191) showed that they are identical and are represented by p191B10 in Fig. 4*C*. The intact *tk* gene of plasmid p191B10 has a 600-bp *Bam*HI–*Nru* I restriction fragment. Therefore, it appears that the 8-bp heterologous insertion in pST8 was excised, and the resulting double-strand gap was repaired to generate the intact *tk* gene in plasmid p191B10.

Table 2. A summary of the effect on recombination frequency of double-strand breaks within the *tk* gene of pSTI plasmids prior to cotransfer with pBS into mouse LTK^- cells

	Relative no. of tk^+ colonies per plate*		
pBS × pSTI*	Uncut pSTI	*Bgl* II-cut pSTI	*Sna*BI-cut pSTI
I = 8	63	206	231
I = 20	56	225	290
I = 24	67	47	284
I = 72	57	44	77
I = 318	47	21	45
I = 611	18	17	52

*I = size of the heterologous insertion in bp.
†See the legend to Table 1.

Double-Strand Breaks Adjacent to Heterologous Insertions. We determined that the effect on recombination frequency of creating a double-strand break outside of the heterologous insertion in pSTI depends on both the location of the double-strand break and the size of the insertion. We previously reported (ref. 10; Table 2) that cutting pSTI 347 bp to the left of the insertion, at the *Bgl* II site, prior to cotransfer with uncut pBS produced a 3- to 4-fold increase in the recombination frequency for insertions less than or equal to 20 bp when compared to uncut pSTI plasmids. There is no beneficial effect of cutting with *Bgl* II for insertions of 24 bp or greater. We concluded that any extension of the double-strand break (the *Bgl* II-cut) into a double-strand gap did not reach the site of the heterologous insertion (a distance of 347 bp); furthermore, the resulting heteroduplex (23) cannot extend through a 24-bp insertion of heterologous DNA.

In the present experiments, we cut the pSTI plasmids 52 bp to the left of the insertion site, at the *Sna*BI site, prior to cotransfer with uncut pBS. In this case, there is a beneficial effect of cutting pSTI at the *Sna*BI site for insertions less than or equal to 24 bp compared to uncut pSTI plasmids. This beneficial effect decreases with insertions of 72 bp or greater (Tables 1 and 2). Therefore, a double-strand break at the *Sna*BI site of pST24 can be extended into a double-strand gap that encompasses the 24-bp heterologous insert to allow a recombination event with pBS that generates an intact *tk* gene. If we assume that the double-strand gap expands symmetrically from the originating double-strand break, then the gap must be at least 152 bp. However, a double-strand break at the *Sna*BI site of pST72 requires the formation of a 248-bp double-strand gap to encompass the heterologous insertion. The mouse L cells are not able to generate and then repair a double-strand gap of 248 bp or larger (Table 2).

DISCUSSION

The cotransfer of pairs of DNAs containing complementary mutations in a gene for a dominant selectable marker into cultured mammalian cells has become a model system to study homologous recombination. Altering the structure of the donor molecules and then scoring for the number of

colonies surviving in a selection medium is a means of analyzing the pathways of homologous recombination. Using different cell types and different selectable markers, we (6, 10) and others (7–9) have observed that the creation of certain double-strand breaks and double-strand gaps in plasmids prior to cotransfer increases the frequency of recombination compared to uncut plasmids. The present study confirms that certain double-strand breaks and a 104-bp double-strand gap are recombinogenic in our system. Therefore, double-strand breaks and double-strand gaps perhaps represent intermediate structures in the pathway of homologous recombination in mammalian cells.

Additional studies have observed that the majority of homologous recombination events that generate an intact gene are consistent with nonreciprocal exchange of DNA or gene conversion. Folger *et al.* (12) demonstrated homologous recombination between pairs of plasmids containing point mutations separated by 417 bp in the neomycin-resistance gene that were microinjected into mouse L cells. Using plasmid rescue, they cloned many examples of the recombined wild-type gene, but they never cloned a double-point mutant that would be expected in a reciprocal exchange reaction. Using a different experimental approach, Liskay and Stachelek (24, 25) stably introduced a plasmid containing two mutant genes into mouse L cells and scored for intrachromosomal homologous recombination. Southern blotting of the recombined cell lines revealed that the majority of recombined cell lines contained one wild-type gene and one single-mutant gene and not one wild-type gene and one double-mutant gene as expected in an intrachromatid reciprocal exchange.

The present study demonstrates that double-strand gap repair, which is a nonreciprocal exchange or gene conversion, is a major pathway in the homologous recombination of cotransferred plasmids in cultured mammalian cells. This conclusion can explain the two important observations made in previous work—i.e., double-strand breaks and gaps are recombinogenic, and homologous recombination usually occurs as a nonreciprocal exchange of DNA. Our most straightforward data supporting gap repair is provided by the cotransfer of uncut pBS with pERV containing a 104-bp double-strand gap. Southern blotting revealed that five of six cloned cell lines had a restriction fragment that was characteristic of a gap repair event, and five of six wild-type rescued plasmids contained the filled-in gap without any other recombination between markers flanking the gene.

By choosing different sites for creating double-strand breaks, we are now able to direct the outcome of homologous recombination between pairs of cotransferred DNAs in cultured mammalian cells. The best demonstration of this result is a comparison of the products of homologous recombination that generate an intact *tk* gene from the cotransfer of pBS with either *Bgl* II-cut pERV or *Eco*RV-cut pERV (Figs. 3 and 4). When pERV is cut with *Bgl* II, a double-strand break is created 286 bp 5′ of the deletion in *tk*. When *Bgl* II-cut pERV is cotransferred with pBS, the predominant product has undergone exchange of flanking markers with the 5′ end coming from pBS and the 3′ end from pERV. In contrast, when pERV is cut with *Eco*RV, the deletion in the *tk* gene is converted to a double-strand gap. When *Eco*RV-cut pERV is cotransferred with pBS, the predominant product has an accurately repaired double-strand gap without exchange of flanking markers.

There are at least two possible explanations for the failure of the cotransfer of pBS and *Bgl* II-cut pERV to produce an intact *tk* gene by gap repair of the deletion of pERV: (*i*) the mouse L cell may not be able to generate and then repair a double-strand gap large enough to encompass the deletion (the distance from the *Bgl* II site to the deletion site is 286 bp; also see below), and (*ii*) when the double-strand gap expands from the *Bgl* II site in a leftward direction, it may extend into the plasmid vector sequence of pERV, which is not homologous with pBS and, therefore, cannot undergo gap repair (see ref. 16).

We also estimated the size of the double-strand gap that the L cell can generate and then repair. We created a double-strand break at the *Sna*BI site of the pSTI plasmids, which is 52 bp to the left of the heterologous insertion in the *tk* gene (Fig. 1*A*). When the *Sna*BI-cut pSTI plasmid was cotransferred with uncut pBS, the double-strand break had a recombinogenic effect for insertions less than or equal to 24 bp but not for insertions larger than 72 bp (Tables 1 and 2). By assuming that the double-strand break at the *Sna*BI site is the site of initiation of a double-strand gap that expands symmetrically, we estimate that the L cell can generate and repair a double-strand gap between 152 and 248 bp.

Note Added in Proof. Liskay and Stachelek (26) have reported that individual intrachromosomal gene conversion events in L cells appear to involve contiguous regions of DNA that are often less than 358 bp in length. This length is comparable to that reported here for the size of the double-strand gaps that an L cell can generate and repair—between 150 and 250 bp.

We thank M. Hsieh and S. Kato for valuable advice and discussion.

1. Miller, C. K. & Temin, H. M. (1983) *Science* **220**, 606–609.
2. Pomerantz, B. J., Nanjokas, M. & Hassel, J. A. (1983) *Mol. Cell. Biol.* **3**, 1680–1685.
3. Shapiro, G., Stachelek, J. L., Letsou, A., Soodak, L. K. & Liskay, R. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4827–4831.
4. Small, J. & Scangos, G. (1983) *Science* **219**, 174–176.
5. Subramani, S. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 1040–1052.
6. Brenner, D. A., Kato, S., Anderson, R. A., Smigocki, A. C. & Camerini-Otero, R. D. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49**, 151–160.
7. Kucherlapati, R. S., Eves, E. M., Song, K.-Y., Morse, B. S. & Smithies, O. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3153–3157.
8. Lin, F. W., Sperle, K. & Sternberg, N. (1984) *Mol. Cell. Biol.* **4**, 1020–1034.
9. Smith, A. J. H. & Berg, P. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49**, 171–182.
10. Brenner, D. A., Smigocki, A. C. & Camerini-Otero, R. D. (1985) *Mol. Cell. Biol.* **5**, 684–691.
11. Folger, K. R., Wong, E. A., Wahl, G. & Capecchi, M. R. (1982) *Mol. Cell. Biol.* **2**, 1372–1387.
12. Folger, K. R., Thomas, K. & Capecchi, M. R. (1985) *Mol. Cell. Biol.* **5**, 59–69.
13. De Saint Vincent, B. R. & Wahl, G. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2001–2006.
14. Orr-Weaver, T. L., Szostak, J. W. & Rothstein, R. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6354–6358.
15. Orr-Weaver, T. L. & Szostak, J. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4417–4421.
16. Szostak, J. W., Orr-Weaver, T. L., Rothstein, R. J. & Stahl, F. W. (1983) *Cell* **33**, 25–35.
17. Enquist, L. W., Vande Woude, G. F., Smiley, J. R. & Summers, W. C. (1979) *Gene* **7**, 335–342.
18. Wigler, M., Pellicer, A., Silverstein, S. & Axel, R. (1978) *Cell* **14**, 725–731.
19. Anderson, R. A., Krakauer, T. & Camerini-Otero, R. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2748–2752.
20. Anderson, R. A., Kato, S. & Camerini-Otero, R. D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 206–210.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–518.
22. Perucho, M., Hanahan, D., Lipsich, L. & Wigler, M. (1980) *Nature (London)* **285**, 207–210.
23. Meselson, M. S. & Radding, C. M. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 358–361.
24. Liskay, R. M. & Stachelek, J. L. (1983) *Cell* **35**, 157–165.
25. Liskay, R. M., Stachelek, J. L. & Letsou, A. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49**, 183–190.
26. Liskay, R. M. & Stachelek, J. L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1802–1806.