MOLECULAR AND CELLULAR BIOLOGY, Apr. 1985, p. 714–720
0270-7306/85/040714-07$02.00/0
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Vol. 5, No. 4

# Homologous Recombination Catalyzed by Human Cell Extracts

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Received 28 September 1984/Accepted 19 December 1984

**Two plasmids containing noncomplementing and nonreverting deletions in a bacterial phosphotransferase gene conferring resistance to neomycin (Neo$^r$) were incubated with human cell extracts, and the mixtures were used to transform recombination-deficient (*recA*$^-$) *Escherichia coli* cells. We were able to obtain Neo$^r$ colonies at a frequency of $2 \times 10^{-3}$. This frequency was 100 to 1,000 times higher than that obtained with no extracts. The removal of riboadenosine 5′-triphosphate, $Mg^{2+}$, or deoxynucleoside triphosphates from the reaction mixture severely reduced the yield of Neo$^r$ colonies. Examination of plasmid DNA from the Neo$^r$ colonies revealed that they resulted from gene conversion and reciprocal recombination. On the basis of these results, we conclude that mammalian somatic cells in culture have the enzymatic machinery to catalyze homologous recombination in vitro.**

Much of our current understanding of the mechanisms of homologous recombination comes from studies of procaryotic systems and fungi. Bacterial systems proved extremely useful, not only because of the excellent genetics but also because it is possible to develop in vitro assays for recombination. For example, the identification of *recA* mutants (6), the development of in vitro assay systems (21), and the purification of *recA* protein (18, 25) have enabled us to understand the pleiotropic effects of *recA*, which plays an important role in the initiation of pairing and strand exchange leading to genetic recombination (for a review, see reference 23). Similarly, study of fungal systems proved fruitful because it is possible to isolate and examine all of the products of a single meiosis, each representing a DNA strand at the time of recombination. The recent development of a cell-free system from yeasts which can mediate homologous recombination (31) promises to yield much useful information.

A similar understanding of recombination processes in mammalian systems has lagged behind. Several efforts to detect recombination between cellular genes at the genetic level in somatic mammalian cells proved unsuccessful (3, 26, 33). Although the presence of sister chromatid exchanges in cells from normal individuals and from patients with Bloom's syndrome or in response to treatment with DNA-damaging agents (5) can be considered to be equivalant to homologous recombination, no direct evidence for the ability of somatic cells to mediate such events has been available. Several recent experiments changed this situation. There are two recent reports (4, 34) which provide evidence for mitotic recombination in mammalian cells. Using recombinant plasmids and gene transfer methods, several investigators have recently shown that mammalian cells can mediate homologous recombination (7, 9, 17, 28, 29). In a long-range program to study homologous recombination and use it for targeted integration of DNA sequences into mammalian cells, we have constructed nonreverting deletion mutants in the eucaryotic-procaryotic shuttle vector pSV2Neo (30). When pairs of these deletion plasmids were introduced simultaneously into mammalian cells, we observed that they underwent homologous recombination at high efficiencies (12). We have also shown that the frequency of recombination can be enhanced, on average, 10-fold if one of the plasmids is linearized by cutting at the site of the deletion. These observations prompted us to investigate whether cell extracts from mammalian cell lines would mediate homologous recombination in vitro. We report our success with the experiments described below.

(Preliminary results of this work were presented at a Cold Spring Harbor Symposium, Cold Spring Harbor, N.Y., in May 1984.)

## MATERIALS AND METHODS

**Cells.** Human EJ cells were a kind gift of R. Weinberg. These cells were grown in Dulbecco modified Eagles medium supplemented with 10% fetal calf serum.

**Plasmids.** The plasmid pSV2Neo was constructed and provided by P. J. Southern and P. Berg (30). The method of introducing deletions in this plasmid and the nature of the deletions were described previously (12).

**Preparation of cell extracts.** EJ cells were grown in Dulbecco modified Eagle medium–10% fetal calf serum. Approximately $2 \times 10^8$ to $3 \times 10^8$ cells were washed three times with phosphate-buffered saline and harvested with a rubber policeman. The cells were centrifuged, and the cell pellet was suspended in 20 ml of Tris buffer (20 mM Tris [pH 7.5], 5 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 200 mM sucrose). After 10 min, the cells were recentrifuged and suspended in the Tris buffer without sucrose but with 1 mM phenylmethylsulfonyl fluoride. Nuclei were released from the cells with 20 to 40 strokes in a Dounce homogenizer and collected by centrifugation at $2,000 \times g$. The pellet is referred to as the nuclear fraction, and the supernatant is referred to as the cytoplasmic fraction. NaCl and EDTA were added to the nuclear fraction to make up 500 mM salt and 10 mM EDTA. This fraction was sonicated for 30 s to disrupt the nuclei and then centrifuged at 18,000 rpm for 30 min. The cytoplasmic fraction was similarly treated but was not subjected to sonication. The supernatants were passed through a DEAE-Sepharose column equilibrated with the same 500 mM NaCl-containing buffer, and fractions rich in protein were identified by absorption at 260 nm. $(NH_4)_2SO_4$ (0.313 g) was added per ml of the pooled fractions, and the resulting solution was centrifuged for 10 min at 10,000 rpm. The pellets were redissolved in 1.5 ml of 50 mM Tris–1 mM EDTA–1 mM dithiothreitol and dialyzed against a buffer

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containing 50 mM Tris (pH 7.5), 10 mM β-mercaptoethanol, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 500 mM NaCl, and 10% glycerol. The dialyzed extract was divided into aliquots and frozen quickly for storage.

**In vitro recombination assay.** A 0.5-μg portion of each of the two plasmids was mixed with various amounts of the cell extracts. The reaction mixture contained 20 mM Tris (pH 7.4), 10 mM $MgSO_4$, 120 mM NaCl, 0.001% gelatin, 1 mM ATP, and 100 nM each of the four deoxynucleotide triphosphates. The reaction volume was 100 μl. After incubation of the plasmids for 1 h at 37°C, pronase was added to a concentration of 100 μg/ml. The DNA was purified by phenol extraction and ethanol precipitation, resuspended in the transformation buffer, and used to transform *Escherichia coli* HB 101 or DH1. In all experiments, after reisolation of DNA from the cell extract, 10% of the total amount was used for each transformation.

**Bacterial transformation and manipulations.** The host *E. coli* HB 101 (*recA13*) or DH1 was transformed by the method of Mandel and Higa (14). Plasmid DNA from the bacterial colonies was isolated by an alkaline lysis procedure (15), digested with appropriate restriction endonucleases, and examined by agarose gel electrophoresis.

## RESULTS

**Experimental plan.** The experimental strategy involved (i) the use of two nonoverlapping, noncomplementing, nonreverting deletion mutants (12) in a bacterial aminoglycoside phosphotransferase gene [APH (3′) II], borne on a procaryotic-eucaryotic shuttle vector pSV2Neo (30); (ii) preparation of cell extracts from a human cell line known to have the ability to mediate high levels of homologous recombination between two exogenously introduced plasmids; (iii) incubation of the mutant plasmids with the extract and use of the resulting DNA to transform a $recA^-$ strain of *E. coli* and select for the expression of the phosphotransferase gene by its ability to confer neomycin resistance ($Neo^r$) or kanamycin resistance to the bacteria.

A diagram of the pSV2Neo plasmid along with some of the relevent restriction enzyme sites and the regions of deletions in shown in Fig. 1. One of the deletions spans a 248-base-pair (bp) region involving the 5′ coding sequences of the gene and is referred to as pSV2Neo deletion left (DL). The other deletion removes a 283-bp segment at the 3′ end of the gene and is referred to as pSV2Neo deletion right (DR). Both DL and DR retain the pBR322 replication origin and can confer ampicillin resistance ($Amp^r$) but not $Neo^r$ or kanamycin resistance to bacteria harboring them.

An established human cell line, EJ, derived from a bladder carcinoma, was used as the source of cell extracts. Actively growing cells were harvested, lysed, and separated into nuclear and cytoplasmic fractions. The nuclear contents were released by lysis, and the nucleic acids from both fractions were removed by passage through DEAE-Sepharose columns. The proteins in the flow-through fractions were precipitated by $(NH_4)_2SO_4$ and dialyzed. The resulting extracts were divided into aliquots and quickly frozen for storage.

In the experimental system, equal proportions (0.5 μg each) of the DL and DR plasmids were mixed with the cell extracts under various conditions for 1 h at 37°C. After incubation, the plasmid DNA was purified, and a fraction of it was used to transform *E. coli* HB 101 (*recA13*) or DH1, both of which are deficient in the major pathway for genetic recombination. The bacteria were plated on plates containing ampicillin or neomycin. $Neo^r$ colonies were isolated, retested, and used for further analysis.

**Cell extracts catalyze homologous recombination.** We have conducted experiments in which the input plasmids were circular or one of the plasmids was linearized by cutting at the site of the deletion to generate a "gapped" linear duplex. The presence of such gapped duplexes is known to enhance homologous recombination in vivo in yeast cells (19) as well as in mammalian cells (12). Most of the results presented in this report were from experiments that involved the use of pSV2Neo DL linearized with *Nar*I and pSV2Neo DR which was undigested.

In all experiments, 0.5 μg each of the two plasmids was incubated with the cell extracts, and a small fraction of the DNA (usually <100 ng) was used for bacterial transformation. Incubation of the plasmids with the extracts reduced their ability to transform bacteria, as determined by the yield of $Amp^r$ colonies, by a factor of 10. We have observed that the incubations result in degradation of DNA, which would explain the lowering of transformation efficiencies. In initial experiments we tested the effect of increasing the amounts of nuclear and cytoplasmic extracts on the yield of $Neo^r$ colonies. When a mixture of the substrates incubated with the reaction mixture without extract was used for bacterial transformation, we obtained $Neo^r$ colonies at a frequency of $2 \times 10^{-5}$ to $8 \times 10^{-5}$. However, when each of the plasmids was incubated separately with the cell extract and mixed before transformation, the yield of $Neo^r$ colonies was less than $5 \times 10^{-6}$. We ascribe these differences to the different amounts of functional DNA present in the two experiments. The low level of $Neo^r$ colonies obtained in these experiments is probably the result of recombination mediated by the *recBC* or *recF* pathways in bacteria. The effects of incubation of plasmid mixtures with cell extracts are presented in Table 1. The use of nuclear or cytoplasmic extracts resulted in a higher frequency of $Neo^r$ colonies, although the nuclear extracts showed a higher level of activity. We observed

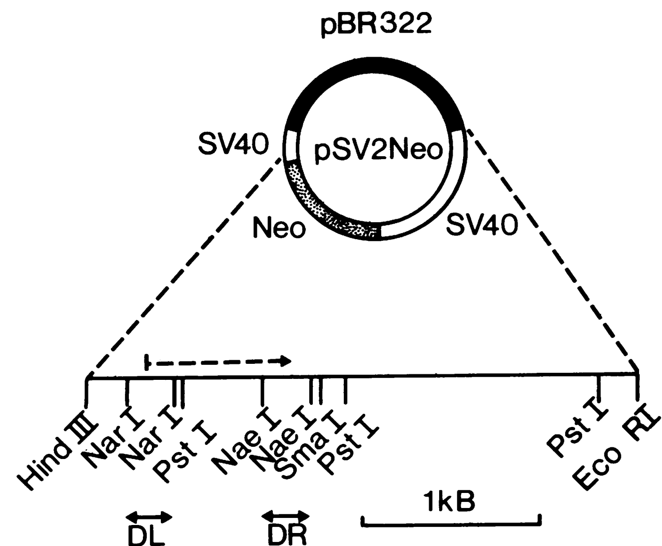


FIG. 1. Structure of the pSV2Neo plasmid. Some of the relevant restriction enzyme sites are shown. The arrow indicates the position of the coding sequence for the phosphotransferase gene. pSV2neo DL was obtained by deleting the 250-bp *Nar*I fragment; pSV2neo DR was obtained by deleting the 280-bp *Nae*I fragment. For more details of the map, see Southern and Berg (30), Kucherlapati et al. (12), and Auerswald et al. (1).

some variability in the yield of Neo$^r$ colonies from different batches of extracts. The maximal yield of Neo$^r$ colonies was $2 \times 10^{-3}$ CFU, which was 100 to 1,000 times greater than that obtained when the two plasmids were incubated separately. The amount of extract that yielded the maximal number of Neo$^r$ colonies varied with the batch of extract. The yield of Neo$^r$ colonies increased with increasing amounts of extract until it reached a maximum and declined with additional amounts of extract. The decline in the yield of Neo$^r$ colonies also coincided with a general reduction in transformation, indicating that the DNA was probably being degraded.

Since the reaction mixture was relatively simple, we have investigated the effects of changing components in the reaction mixture on the yield of Neo$^r$ colonies. Results from these experiments are also shown in Table 1. Lack of riboadenosine 5'-triphosphate and $Mg^{2+}$ in the reaction mixture reduced the yield of Neo$^r$ colonies to 8.5 and 2.4% of that of the control, respectively. When deoxynucleoside triphosphates were removed, we failed to obtain any Neo$^r$ colonies. The use of boiled extracts had a similar effect. Taken together, these results indicate that the Neo$^r$ colonies resulted from the action of one or more components of the cell extract on the plasmid substrates. We observed that the production of recombinants is most efficient when one of the input plasmids is linearized at the site of the deletion (Table 1). These results are consistent with in vivo studies conducted previously (12).

**Products of recombination.** To extend our studies and to confirm that the Neo$^r$ colonies resulted from recombination between the input molecules, we isolated plasmid DNA from several of the Neo$^r$ colonies and examined the DNA by agarose gel electrophoresis, usually after digestion with restriction endonucleases. We first examined the sizes of the molecules obtained from the Amp$^r$ and Neo$^r$ colonies by fractionating undigested plasmid DNA on agarose gels. DNA from colonies selected for Amp$^r$ alone was always monomeric, whereas DNA from Neo$^r$ colonies was monomeric, dimeric, or multimeric. Since *recA*$^-$ cells are incapable of converting monomeric molecules into dimers or dimers into monomers (20), we conclude that the dimers were generated in vitro. The fact that all of the dimeric molecules were Neo$^r$ strongly suggests that the dimerization and acquisition of the Neo$^r$ phenotype originated from the same event involving the extract.

TABLE 1. Effect of changing conditions on the yield of Neo$^r$ colonies

| Plasmid substrate | Conditions | Frequency of Neo$^r$ colonies ($10^{-6}$) | % Maximum |
|---|---|---|---|
| DL(*Nar*I) + DR | No extract | 9.3 | 0.5 |
| DL(*Nar*I) + DR | Separate incubations | <48 | 0 |
| DL(*Nar*I) + DR | 10 μl of extract | 280 | 14 |
| DL(*Nar*I) + DR | 30 μl of extract | 2,000 | 100 |
| DL + DR(*Nae*I)[a] | 30 μl of extract | 130 | 6.5 |
| DL + DR | 30 μl of extract | 4 | 0.2 |
| DL(*Nar*I) + DR | 30 μl of extract; no ATP | 170 | 8.5 |
| DL(*Nar*I) + DR | 30 μl of extract; no $Mg^{2+}$ | 48 | 2.4 |
| DL(*Nar*I) + DR | 30 μl of extract; no dNTPs | <80 | 0 |
| DL(*Nar*I) + DR | 30 μl of boiled extract | <13 | 0 |

[a] The enzyme *Nae*I does not cleave its recognition site 100% of the time. This is recognized by us and the manufacturer (New England BioLabs). This may explain the lower levels of recombination observed in this case.

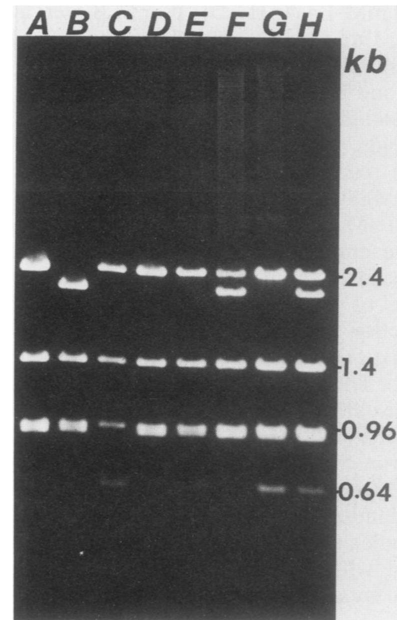


FIG. 2. Nature of the plasmid DNA in Neo$^r$ colonies. Plasmid DNA was isolated by the alkaline lysis procedure, digested with *Pst*I, and fractionated on agarose gels. Lanes: A, pSV2Neo; B, pSV2Neo DL; C, pSV2Neo DR; D through H, DNA from Neo$^r$ colonies—D, wild-type monomer; E, monomeric mixture containing wild-type and DR; F, monomeric mixture containing wild-type and DL; G, dimer containing wild-type and DL; H, multimer containing wild-type, DR, and DL.

To ascertain the nature of the molecules in Neo$^r$ colonies, plasmid DNA from several colonies was digested with restriction endonucleases and examined by gel electrophoresis. Representative results from *Pst*I digestions are shown in Fig. 2, and the complete set of results is summarized in Table 2. *Pst*I is especially suitable because it enables us to distinguish between the wild-type *neo* gene and the two deletion molecules. *Pst*I digestion of pSV2Neo yields four fragments, 2.4, 1.4, 0.96, and 0.92 kilobases (kb) in length (Fig. 2, lane A). If a plasmid carries the DL gene, it is characterized by the presence of a 2.2-kb band (lane B). The presence of a DR molecule can be inferred from the presence of a 0.68-kb band which replaces the 0.92-kb band (lane C). Bacteria that harbored monomeric molecules gave the types of patterns shown in lanes D through F. They correspond to exclusively wild-type molecules (48%, lane D), a mixture of

TABLE 2. Nature of plasmid DNA in Neo$^r$ colonies

| Type of molecule | No. of molecules (%) |
|---|---|
| Monomer | |
| Wild type | 33 (48) |
| Wild type + DR | 15 (23) |
| Wild type + DL[a] | 3 (4) |
| Dimer | |
| Wild type + DR | 14 (20) |
| Wild type + DL | 4 (0) |
| Multimer | 3 (4) |

[a] These plasmids probably arose through interactions between uncut parental molecules. Despite extensive digestions, *Nar*I fails to cleave its recognition sites at all. This observation has been made by us and the manufacturer (New England BioLabs, Inc.). For more explanation, see the text and Fig. 4.

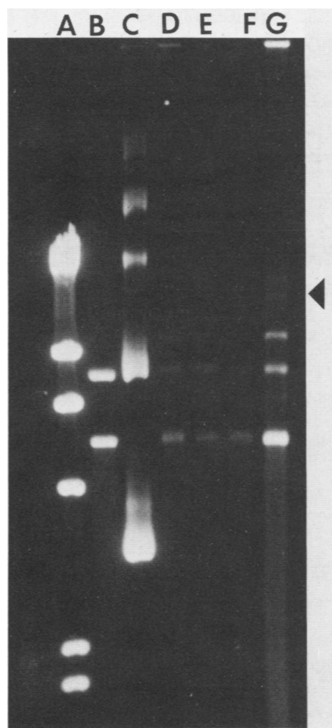


FIG. 3. Results of coincubation of deletion plasmids. After incubation of the substrates with the extract, DNA was isolated and fractionated on agarose gels. Lanes: A, λ *Hin*dIII digest; B, pSV2Neo DL (*Nar*I); C, pSV2Neo DR; D through G, DNA after various periods of incubation—D, 15 min; E, 30 min; F, 45 min; G, 60 min. Arrows indicate novel higher-molecular-weight forms enriched in dimers.

wild-type and DR molecules (23%, lane E), and a mixture of wild-type and DL molecules (4%, lane F). All of the dimeric molecules yielded the pattern shown in lane G, which contains bands derived from a wild-type plasmid and DR (20%). The multimeric molecules, which constituted 4% of the total, gave a pattern (lane H) which indicated that they contain a wild-type gene, DL, and DR. Among the $Neo^r$ colonies, there are four possible dimeric molecules that may result from recombination. Each of the dimers must contain a wild-type gene. In addition, it may contain another normal gene (class 1), a doubly deleted gene (class 2), a DR gene (class 3), or a DL gene (class 4). Of these we detected only class 3 molecules, which constituted 24% of the total. This may be related to the fact that among the input molecules, the DL was linear and the DR was circular (see below and Fig. 4).

Since *recA*$^-$ bacteria have a severely limited ability to convert monomeric molecules into multimeric molecules (20), the high proportion of dimeric molecules among the $Neo^r$ colonies we observed is most likely to be generated in vitro. Since all these molecules contain a wild-type gene and a DR gene, they must have resulted from repair of the deleted region in DL followed by recombination. Thus the dimeric molecules we obtained must have resulted from genetic recombination. Since the bacterial host does not mediate this reaction, we conclude that the reactions leading to the formation of these molecules are catalyzed by enzymes present in the cell extracts.

To further confirm the view that dimeric molecules were generated by the action of components present in the extract, we conducted the following experiments. Linearized pSV2Neo DL and circular pSV2Neo DR were coincubated or incubated separately with the extract, and the resulting DNA was fractionated on agarose gels. Both of the incubations generated forms of DNA which migrated as larger-than-unit-size molecules (Fig. 3). These higher-molecular-weight forms could represent exclusively either catenanes resulting from the action of topoisomerases or dimers resulting from homologous recombination. Detection of dimers in this region would show that recombination events have occurred in vitro. DNA from this region of one such gel was isolated and used to transform *E. coli* DH1. We observed that the DNA derived from the mixture of input plasmids incubated separately gave all monomers, whereas 10% of the molecules from the coincubation were dimers. These results clearly indicate that events leading to the formation of dimers were already completed before the bacterial transformation. The remaining high-molecular-weight forms that produce monomers on transformation are presumed to be catenanes of pSV2Neo DR formed by topoisomerase activity. Since the linearized pSV2Neo DL is unable to participate in this reaction but is at least 100 times more efficient at producing genetic recombinants than is the circular form, catenation does not contribute to the recombination observed.

## DISCUSSION

We have presented evidence which indicates that cell extracts from a human cell line are capable of catalyzing homologous genetic recombination between two bacterial plasmids. Several different lines of evidence support this conclusion. We and several other investigators (7, 9, 12, 17, 28, 29) have shown that somatic mammalian cells have the enzymes needed for recombination between exogenously introduced plasmids and that this process occurs at high efficiency. It is therefore to be expected that such cells will contain the enzymes needed to mediate the reaction in vitro. It is well established that *recA*$^-$ bacterial cells have a severely reduced ability to mediate homologous recombination (6). The parental molecules (reaction substrates) have extensive deletions and are incapable of reversion (12), and coincubation of the two plasmids is needed to yield colonies representing recombinant DNA molecules. By showing that coincubation of plasmids is necessary to yield any recombination products, we have eliminated the possibility that the extracts are capable of inducing alterations in the plasmids which may enhance their ability to undergo recombination in the *recA*$^-$ bacteria.

Strong evidence that homologous recombination was mediated by components present in the cell extracts was provided by the following facts. (i) Accumulated results from a number of experiments showed that the maximal recombination frequency we observed is about $2 \times 10^{-3}$, which is 100 to 1,000 times greater than that obtained without the use of extracts or when the two plasmids were incubated separately. (ii) The production of recombinants is abolished if each of the two substrates is incubated with the extract separately; this indicates that the reaction involves an interaction between the two parental molecules in vitro rather than a potentiation of the individual parents, for example, the simple introduction of gaps or nicks for bacterially mediated events. (iii) The yield of recombinant colonies was severely reduced or eliminated if the reaction mixtures did not contain riboadenosine 5′-triphosphate, $Mg^{2+}$, or deoxynucleoside triphosphates; boiling the extract before incubation of the plasmids also yielded similar results. (iv) Examination of the DNA from the $Neo^r$ colonies revealed that a substantial proportion of the plasmids (24%) are dimers. Potter and Dressler (20) have shown that *recA*$^-$

*E. coli* strains are incapable of mediating the formation of dimeric molecules from monomeric molecules and vice versa. None of the Amp$^r$ colonies tested contained dimeric molecules. Isolation of DNA which was larger than unit size from the coincubation yielded a high proportion of dimeric molecules. On the basis of these lines of evidence, we conclude that mammalian cell extracts can initiate and catalyze homologous recombination.

Cell extracts from other systems have been used to examine homologous recombination. Keene and Ljungquist (11) have purified a human protein which promotes D-loop formation, a presumed recombinational intermediate. Bacterial cell extracts have been shown to be quite effective (13, 21, 27), yielding recombination frequencies of up to 0.1% per kb of homology. Similar results have been obtained by Symington et al. (31), using yeast cell extracts. The recombination frequencies we have observed (0.4% per kb of homology) compare favorably with these results.

The exact nature of the reactions mediated by the extracts can only be supposed. Since strand exchange is an important feature of initiation of recombination, one or a complex of enzymes that mediate this reaction, the equivalent of a bacterial *recA*-like activity is probably present in the extracts. The requirement for deoxynucleoside triphosphates in the recovery of Neo$^r$ colonies and the fact that the gap of 248 bp is repaired in the process of recombination implicate a DNA polymerase activity. Other activities, such as a topoisomerase, a ligase, and other enzymes which may be needed to resolve certain recombination intermediates, may be present in the extract or are supplied by the bacteria. In independent tests we were able to determine that in addition to nuclease activity, the extracts exhibited DNA polymerase and topoisomerase activity. It is possible that the in vitro system carries out some of the initial steps of recombination and the remaining steps are completed in the bacterial host.

Examination of recombination products from our experiments provided valuable information about the mechanism by which these products were generated. Analysis of the dimeric molecules was most instructive because they retain the reciprocal products of recombination. All of the dimeric molecules we obtained from the incubation of linear DL and circular DR contained a wild-type *neo* gene and a DR *neo* gene. This product must have been generated by some mechanism which repaired the gap at the DL deletion site, accompanied by recombination. Bacteria that harbored two monomeric molecules, one wild type and another DR, could have resulted from cotransformation of bacteria or transformation with a heteroduplex DNA containing wild-type and DR strands. It is unlikely that they have resulted from cotransformation because of their high frequency (27%) and because examination of DNA from Amp$^r$ colonies revealed that they are uniformly monomeric and of one class, predominantly DR. The heteroduplex molecule could have resulted from gap repair followed by branch migration across the deleted region. That such branch migrations can occur has been postulated by Radding (22). Results presented by Fink and Styles (8) and Bianchi and Radding (2) show this to be the case. An alternative possibility is that these bacteria have resulted from transformation by an unresolved recombination intermediate (see Fig. 4c). We cannot rigorously exclude this possibility. The low level of wild-type and DL mixtures in this experiment can be explained by interactions between two uncut parental molecules (see legend to Table 2).

Several models of homologous recombination have been proposed. The most widely accepted among these is that

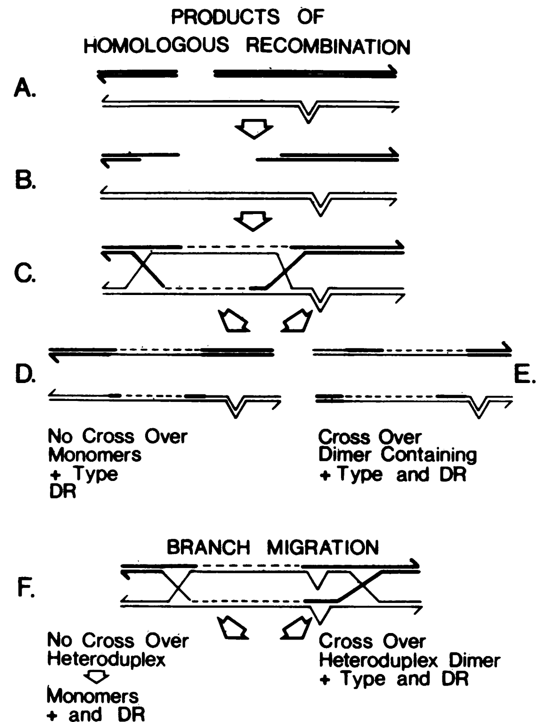


FIG. 4. Possible mechanism for the origin of the plasmids observed in the Neo$^r$ colonies. For the sake of simplicity, the DNA molecules are represented as being linear. The model is the double-strand repair model of recombination proposed by Szostak et al. (32).

proposed by Meselson and Radding (16), which in turn was based on a model by Holliday (10). Resnick and Martin (24) have noted that double-stranded breaks can be repaired by a process involving recombination. Orr-Weaver et al. (19) have shown that homologous recombination between yeast chromosomal genes and their homologs on a plasmid can be enhanced as much as 1,000-fold by introducing double-stranded breaks in the input plasmid within the region of homology. On the basis of these and other observations, Szostak etal. (32) have proposed a recombination model which is referred to as a double-strand break repair model of recombination. This model is different from that proposed by Meselson and Radding (16) in that it requires two rounds of single-strand repair synthesis, formation of heteroduplex DNA surrounding the gap, and formation and resolution of two Holliday junctions (10). Since the results discussed in this report were obtained from the use of a plasmid containing a double-stranded break, we examined our results to ascertain whether they are consistent with the double-strand break repair model. The possible events leading to gap repair and recombination and the expected products of such a reaction are shown in Fig. 4. Comparison of results summarized in Table 2 are indeed consistent with these expectations. On the basis of this model, the monomeric wild-type molecules would result from gap repair and no crossing-over (Fig. 4D). The gap repair events are equivalent to gene conversion. The mixture of monomeric wild-type and DR molecules would also result from gene conversion, followed by branch migration and no crossing-over (Fig. 4F). The third class, dimeric molecules containing the wild-type and DR genes would result from gene conversion followed by crossing-over with or without branch migration (Fig. 4E and F). It is possible that gene conversion occurs after the

formation of heteroduplex molecules shown in Fig. 4F. Gene conversion of monomeric heteroduplex would result in a wild-type or DR molecule. Gene conversion of one of the two heteroduplexes generated from crossing-over of the molecule shown in Fig. 4F could lead to a bacterium carrying a dimer containing two wild-type genes and a dimer containing a wild-type gene and DR. Two gene conversion events could lead to a dimer containing two wild-type genes. Examination of DNA from a larger sample of Neo$^r$ colonies may permit us to detect these other classes of molecules. The experimental system we have used requires a gene conversion event which may or may not be associated with a crossing-over event to yield phenotypically wild-type molecules. In addition, the recovery of dimeric molecules containing wild-type and DR genes is indicative that in mammalian cell extracts, a double-strand break can initiate homologous recombination events.

Despite several attempts, little or no homologous recombination between homologous cellular genes was detected in somatic mammalian cells (3, 26, 33). As such, it is likely that the enzymes that mediate this reaction in vitro are those that are necessary for normal DNA replication and repair in the cells. If this were indeed the case, our results provide much hope for the genetic and biochemical characterization of these processes. For example, it is possible to examine extracts from cells derived from patients known to have DNA repair defects such as Xeroderma pigmentosum, Bloom's syndrome, and Ataxia telangectasia. If these extracts fail to mediate homologous recombination, extracts from mutants which are known genetically to complement the defects can be mixed and tested, providing a biochemical assay for genetic analysis. If this approach is successful, it should be possible to fractionate the cell extract and add individual fractions to extracts from repair-deficient cells in a complementation assay. We have embarked on a program to test these and other similar possibilities.

## ACKNOWLEDGMENTS

We thank Oliver Smithies for many helpful discussions, and R. Davidson, C. Radding, S. Ross, A. Skoultchi, and V. Zakian for comments on the manuscript. Linda Cardenas typed the manuscript. We acknowledge technical support from L. Wallace. D. Clark did the experiments whose results are shown in Fig. 3.

This work is supported by a Public Health Service grant from the National Institutes of Health and a grant from the March of Dimes Birth Defects Foundation to R.K.

## ADDENDUM

While this manuscript was in the review process, a report by Darby and Blattner (Science 226:1523, 1984) appeared, the results of which support our view that mammalian cell extracts can catalyze homologous recombination between two DNA molecules.

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