HOMOLOGOUS RECOMBINATION BETWEEN AUTONOMOUSLY REPLICATING PLASMIDS IN MAMMALIAN CELLS

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ABSTRACT

The ability of autonomously replicating plasmids to recombine in mammalian cells was investigated. Two deletion plasmids of the eukaryotic-prokaryotic shuttle vector pSV2neo were cotransfected into transformed monkey COS cells. Examination of the low molecular weight DNA isolated after 48 hr of incubation revealed that recombination between the plasmids had occurred. The DNA was also used to transform *recA*$^-$ *E. coli*. Yield of neo^R colonies signified homologous recombination. Examination of the plasmid DNA from these colonies confirmed this view. Double-strand breaks in one or both of the input plasmids at the sites of deletion resulted in an enhancement of recombination frequency. The recombination process yielded monomeric and dimeric molecules. Examination of these molecules revealed that reciprocal recombination as well as gene conversion events were involved in the generation of plasmids bearing an intact neo gene. The COS cell system we describe is analogous to study of bacteriophage recombination and yeast random-spore analysis.

MUCH of our understanding of the mechanism of genetic recombination comes from study of bacteria and fungi. In bacteria, the combination of genetic methods and *in vitro* recombination systems has permitted identification of several loci and enzymes, notably recA protein, that play an important role in recombination. The ability to recover each and all of the products from a single meiosis in fungi has provided an excellent opportunity to study mechanisms of recombination in these organisms. Similar studies of mammalian recombination were hampered by the lack of an appropriate system to study these processes. Several early attempts to detect mitotic recombination in mammalian systems failed, but there is now ample evidence that somatic mammalian cells have all the enzymatic machinery needed to mediate homologous recombination. WASMUTH and VOCK HALL (1984) obtained evidence for mitotic recombination. In another class of experiments, two plasmids or viral DNA molecules, each containing a mutant or deleted gene, were introduced simultaneously into mammalian cells, and recombination was monitored by the recovery of colonies or plaques that resulted from the presence of a functional gene (WAKE and WILSON 1979; UPCROFT, CARTER and KIDSON 1980; FOLGER

et al. 1982; DE SAINT VINCENT and WAHL 1983; SMALL and SCANGOS 1983; SHAPIRA *et al.* 1983; MILLER and TEMIN 1984; VOLKERT and YOUNG 1983; KUCHERLAPATI *et al.* 1984b). In some cases, the recombinant products can be rescued from the cells and subjected to detailed analysis (KUCHERLAPATI *et al.* 1984b). These methodologies are time-consuming, and only a limited number of recombinant products can be examined at the molecular level. We have developed an assay system that is rapid and enables us to examine hundreds of recombination products in a relatively short period of time. Results obtained from this system are presented here.

The experimental system utilizes three important features: (1) the use of pSV2neo, a prokaryotic-eukaryotic shuttle vector plasmid (SOUTHERN and BERG 1982); (2) monkey COS cells (GLUZMAN 1982), a cell line in which the vector can recombine and replicate autonomously; and (3) a *recA*$^-$ bacterial strain that is deficient in the major pathway for recombination but acts as a reporter system by permitting us to detect recombination events that have occurred in a mammalian cell. These three features make this system analogous to infection of bacteria with appropriate bacteriophage, which can recombine and replicate autonomously in the host cell. A preliminary report of this work was presented at the Cold Spring Harbor Symposium held during May–June 1984 (KUCHERLAPATI *et al.* 1984a), and while this manuscript was in its final stages of preparation, another paper appeared describing the feasibility of using COS cells to study homologous recombination (RUBNITZ and SUBRAMANI 1985).

MATERIALS AND METHODS

Cells: Monkey COS cells (GLUZMAN 1982) are a gift from K. SUBRAMANIAN at our institution. They are SV40 transformed monkey cells that permit autonomous replication of plasmids containing an SV40 origin of replication. They are grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum.

Plasmids: pSV2neo (SOUTHERN and BERG 1982) is a plasmid containing a bacterial replication origin and a bacterial amido-phosphotransferase gene [AP (3′) II], which confers bacteria resistance to the antibiotics neomycin or kanamycin. This gene can also be expressed in mammalian cells by virtue of being under the control of SV40 early gene transcriptional signals. The construction of two pSV2neo deletion plasmids was described (KUCHERLAPATI *et al.* 1984b) and the structure of the plasmids shown in Figure 1. pSV2neo DL (DL) contains a 248 base pair (bp) deletion spanning the 5′ end of the neo gene. pSV2neo DR (DR) carries a 283 bp deletion spanning the 3′ end of the neo gene. Each of these deletions renders the neo gene inactive.

In some experiments we used a modified pSV2neo DR. The coding region for the neo gene in DR is flanked by a *Hin*dIII site at the 5′ end, a *Nae*I site at the site of the DR deletion and a *Sma*I site at the 3′ end. These three sites were changed to *Sma*I, *Sal*I and *Xba*I, respectively, by using appropriate linkers. The methods used to change the restriction enzyme sites were described by MANIATIS, FRITSCH and SAMBROOK (1982). This plasmid is referred to as pSV2neo DR SSalX. The alteration of these restriction sites does not have any effect on the overall structure and function of the plasmid. The polymorphism in restriction enzyme sites present on DL and DR SSalX were used to determine the relative contributions of gene conversion and reciprocal recombination in the generation of plasmids containing a wild-type neo gene.

Transfections: In initial experiments, DNA transfections were achieved by a calcium phosphate coprecipitaton method (LOWY, RANDT and SCOLNICK 1978). In this method, 10^6 COS cells were plated in a 100-mm dish. The following day a calcium-phosphate precipitate of carrier-free DNA was prepared. In these experiments, 1 μg of each of the deletion plasmids was mixed and precip-

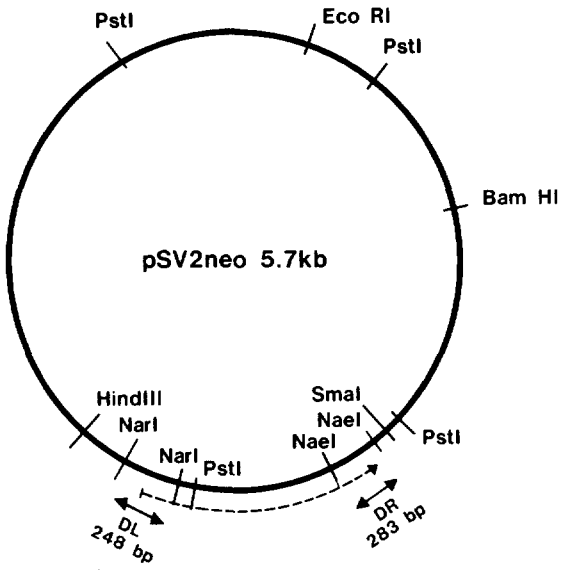

FIGURE 1.—Structure of pSV2neo plasmid and the derivative deletions. The position of some restriction enzyme sites are indicated. Broken line represents the DNA from Tn*5* carrying the neo gene. The lines with the double arrows indicate the regions deleted in pSV2neo DL and pSV2neo DR. For details about construction of these plasmids, see SOUTHERN and BERG (1982) and KUCHERLAPATI *et al.* (1984b).

itated, and the precipitate was added to cells. Four hours later, the medium was removed and the cell sheet was treated with 10% dimethyl sulfoxide in Hanks' balanced salts for 2 min. In later experiments DEAE-dextran-mediated transfer (SUSSMAN and MILMAN 1984; LOPATA, CLEVELAND and SOLLNER-WEBB 1984) was used to introduce DNA into cells. In this method, 8 μg each of the two deletion plasmids were mixed with DEAE-dextran (200 μg/ml in serum-free medium) and layered onto a plate of 10^6 cells. The cells were washed after 4 hr and treated with 10% dimethyl sulfoxide, as described above. The low molecular weight (MW) DNA was isolated from the COS cells after 48 hr of incubation (unless otherwise indicated) and was used for direct analysis or for transformation of *recA*$^-$ strains of *E. coli.* The recovery of DNA was measured by the yield of amp^R colonies. We noted that the yield of amp^R colonies per microgram of input DNA was 3–6 times greater with the DEAE-dextran method than with the $CaPO_4$ method.

DNA manipulations: Low MW DNA from COS cells was isolated by the method of HIRT (1967). This DNA was used directly for digestion with different restriction endonucleases, or was used to transform bacterial *recA*$^-$ strains HB101 or DH1 by the method of MANDEL and HIGA (1970), as modified by MANIATIS, FRITSCH and SAMBROOK (1982). Plasmid DNA from bacteria was isolated by an alkaline lysis procedure (MANIATIS, FRITSCH and SAMBROOK 1982). DNA was digested with appropriate restriction endonucleases according to directions provided by the manufacturer (New England Biolabs) and was fractionated on 1% agarose gels. When necessary, DNA was transferred to nitrocellulose by the method of SOUTHERN (1975) and was blot-hybridized with appropriate labeled probes. DNA for hybridization was labeled by a random primer extension method, described by FEINBERG and VOGELSTEIN (1983).

RESULTS

Experimental strategy: Deletion plasmids pSV2neo DL and DR or its derivative DR SSalX were simultaneously introduced into monkey COS cells by DNA transfection methods. Forty-eight hours later, low MW DNA was isolated from

TABLE 1

Recombination of pSV2neo deletion plasmids in monkey COS cells

Substrates	neo^R/amp^R colonies[a]	Frequency $\times 10^{-3}$
pSV2neo DL	0/22,680	<0.04
pSV2neo DR	0/117,600	<0.009
pSV2neo DL + DR (separate transfections)[b]	0/72,450	<0.01
pSV2neo DL + DR	614/155,820	3.9

[a] Bacterial transformation resulted in 2 ml of culture. 10–100 μl of this culture was used to deduce the total number of amp^R colony-forming units. The remaining culture was used to obtain the number of neo^R colonies.

[b] COS cells were transfected with DL or DR alone. Low MW DNA from each of these experiments was mixed and used to transform *E. coli.*

the transfected cell population and used for direct analysis or for bacterial transformation. Recombination frequency is expressed as the ratio of the number of bacterial colonies that can grow in neomycin to those that can grow in ampicillin.

Recombination in COS cells: To ascertain if recombination occurs in COS cells, a mixture of pSV2neo DL and DR was introduced into monkey cells, and low MW DNA was isolated after 48 hr, as described above. This DNA was used to transform *recA*$^-$ *E. coli* strains HB101 or DH1. Results of this experiment are shown in Table 1. Amp^R colonies represent parental plasmids, whereas neo^R colonies represent recombinant plasmids. We were able to obtain neo^R colonies at an average frequency of 3.9×10^{-3}. As each of the parental plasmids carries a deletion of the neo gene, they cannot undergo reversion. The only possible way that a wild-type neo gene can be generated is through recombination involving the two deletion plasmids.

To ascertain that the neo^R colonies have resulted from recombinational events that occurred in mammalian cells, rather than in the bacteria, we have conducted several different experiments. A mixture of DL and DR, which was not passaged through COS cells, gave neo^R colonies at a frequency that is at least 100 times lower than that obtained with low MW DNA from COS cells. When DL and DR were separately transfected into COS cells, low MW DNA isolated, mixed and used to transform bacteria, the yield of neo^R colonies was again 100 times lower (Table 1). To confirm that recombination has occurred in COS cells, we conducted the following experiment. Low MW DNA isolated from transfection of DL and DR SSalX mixtures was cut with appropriate restriction endonucleases and was blot-hybridized with a neo gene probe. Results of this experiment are shown in Figure 2. Digestion of pSV2neo with *Hin*dIII-*Sma*I yields 4.4 and 1.3 kb bands; the 1.3 kb band contains the neo gene. The corresponding regions in DL and DR are 1.06 and 1.04 kb in length. The presence of a 1.3 kb band in the DNA from the transfected cells is a clear indication that recombination has occurred in the COS cells. Such a 1.3 kb band is, indeed, detectable in DNA isolated from COS cells (Figure 2, lanes D–E). These results are most consistent with the view that homologous

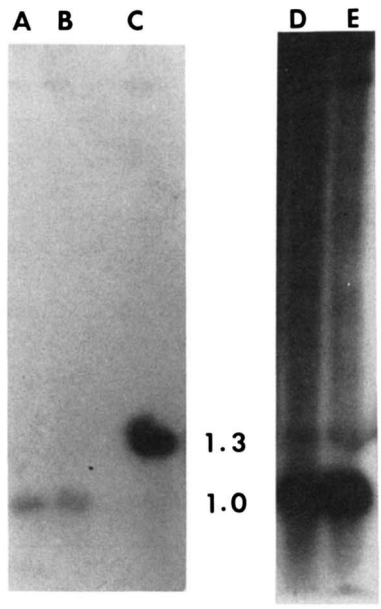

FIGURE 2.—Test for recombination in COS cells. DL and DR SSalX were cotransected into monkey cells, low MW DNA isolated, digested with appropriate restriction endonucleases and blot-hybridized using a neo-specific probe. Lane A, Low MW DNA digested with *Hin*dIII-*Sma*I; lane B, Low MW DNA digested with *Sma*I-*Xba*I; lane C, pSV2neo digested with *Hin*dIII-*Sma*I. Lanes D and E are longer exposures of lanes A and B.

recombination between input plasmids occurs in COS cells, and the bacteria act only as a reporting or recording system for these events.

We have ascertained whether multiple rounds of recombination events occur in COS cells. Cells were transfected with a mixture of DL and DR, and low MW DNA was isolated after various time points and was used to transform bacteria. Results of this experiment are shown in Figure 3. If recombination were restricted to a specific time after transfection, neo^R colonies would be recovered from DNA obtained after a specific time period, and the frequency would remain unchanged for the rest of the period. If multiple rounds of recombination occurred, the frequency of neo^R colonies would be expected to increase during the 48-hr period. The results we have obtained indicate that the recombinational events are occurring throughout the 48-hr period tested.

To test if DNA replication is necessary for recombination, we have transfected monkey CV1 cells with the pair of deletion plasmids. CV1 cells do not contain SV40 T-antigen and, as such, cannot support the replication of pSV2 plasmids. We were able to obtain neo^R colonies from DNA isolated from these cells (8 neo^R/18,990 amp^R), indicating that DNA replication is not a prerequisite for homologous recombination in mammalian cells.

We have also tested the effect of double-strand breaks on the recombination in COS cells. It has been shown that introduction of double-strand breaks in the region of homology enhances homologous recombination in yeast (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981), as well as in mammalian cells (KUCH-

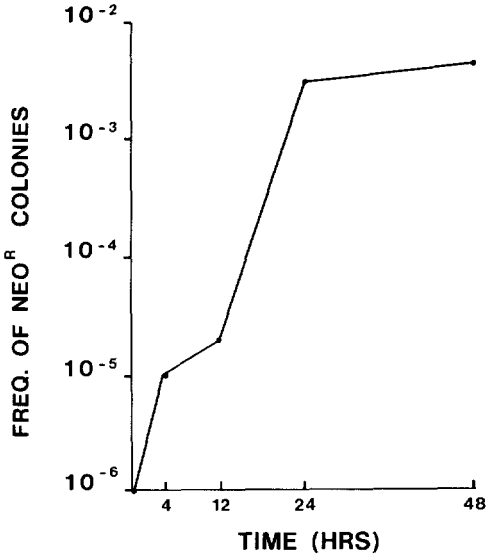

FIGURE 3.—Kinetics of the yield of neo^R colonies derived from DNA passaged through COS cells. Experimental data from five kinetic experiments, each of which assayed the frequencies of neo^R recombinants at 4, 12, 24 and 48 hr posttransfection, were pooled to prepare this figure.

TABLE 2

Effect of double-strand cuts on homologous recombination in COS cells

Experiment	pSV2neo DL digestion	pSV2neo DR digestion	Frequency neo^R/amp^R × 10^{-5}	Ratio
1				
			2.35 ± 0.15	1
	*Nar*I		5.6 ± 0.10	2.3
	*Eco*RI		1.1	
2[a]				
			4.7 ± 1.3	1
		*Sal*I	28 ± 6	6.0
3[a]				
			5.5	1
		*Sal*I	39	7
	*Nar*I	*Sal*I	320	58

[a] In these sets of experiments, pSV2neo DR SSalX was used.

ERLAPATI *et al.* 1984b). Accordingly, pSV2neo DL was digested with *Nar*I and pSV2neo DR SSalX was digested with *Sal*I, each of which cuts at the respective deletion sites. Each of the linear molecules was mixed with its appropriate partner in its circular form and was cotransfected into COS cells. Results of a representative set of experiments are presented in Table 2. Cutting one or both of the input molecules within the region of homology enhanced (2–58 times) the yield of neo^R colonies. When one of the molecules was cut outside the region of homology (*Eco*RI) there was no detectable increase in recombi-

nation. These results indicate that double-strand breaks may play an important role in recombination.

Nature of recombinant products: To understand the nature of recombination, we have examined the plasmids harbored by the neo^R bacteria. Plasmid DNA was isolated from neo^R bacteria and fractionated on agarose gels. The classes and frequencies of different types of molecules are shown in Table 3. The plasmids were of four categories. One class is of a monomeric size, a second class of dimeric size and the other two classes were smaller than both monomer and dimer. When they were smaller than unit size, their size varied considerably. It is known that a high proportion of autonomously replicating plasmids have a tendency to suffer deletions (CALOS, LEBKOWSKI and BOTCHAN 1983; RAZZAQUE, MIZUSAWA and SEIDMAN 1983; RAZZAQUE *et al.* 1984; LEBKOWSKI *et al.* 1984; ASHMAN and DAVIDSON 1984). The smaller than unit size molecules can be explained to have been derived from such events.

We have also examined the plasmids following digestion with restriction endonucleases (Figure 4). The immediate products of recombination between two circular molecules could be either monomers or dimers. The dimers that are formed in COS cells are expected to have the ability to resolve themselves into their constituent monomers through additional rounds of recombination. Indeed, 238 of 332 (72%) of all the neo^R colonies harbored monomeric or modified monomeric molecules. Because the neo gene in DL and DR SSalX is flanked by diffeent restriction enzymes sites, these sites can be used as polymorphic genetic markers to examine the nature of recombination. Results of such an examination are summarized in Table 4. DL contained a *Hin*dIII site at its 5′ end and a *Sma*I site at the 3′ end. DR SSalX contained a *Sma*I site at its 5′ end and an *Xba*I site at its 3′ end. When neither DL nor DR SSalX were cut, three types of monomeric molecules were observed. These contained (1) a *Hin*dIII and a *Sma*I site, (2) a *Sma*I and an *Xba*I site and (3) those that contained two *Sma*I sites. The first two categories could result from gene conversion or double crossover events, whereas the third category could result from a reciprocal recombination or coconversion of a deleted region and its most proximal non-*Sma*I site. If gene conversion plays an important role, it should be reflected in the larger than monomeric and dimeric molecules. Gene conversion of one of the deleted plasmids, followed by crossing over, would result in a dimer containing a wild-type gene and DL (class I) or DR (class II). Recombination without conversion would result in a molecule containing a wild-type gene and a doubly deleted gene (class III). Examination of 14 such molecules revealed that four were of class I, four of class II and five of class III, and one was of indeterminate type. The relatively large proportion of dimeric molecules containing a wild-type and a deleted neo gene indicates that gene conversion resulting from gap repair plays an important role in the generation of a wild-type neo gene. This view was substantiated when the monomers obtained from a DL + DR SSalX (digested with *Sal*I) cross were examined. Only two of the three types of monomeric molecules obtained from the DL + DR cross were obtained (Table 4). From these results we conclude that molecules containing *Sma-Xba*I sites have resulted from con-

TABLE 3

Classes of recombinant molecules

Substrates	No. of plasmids (%)					
	Total	Monomer	<Monomer	Dimer	<Dimer	Multimer
DL + DR	161	109 (68)	8 (4)	12 (7)	24 (15)	2 (1)
DL (*Eco*RI)[a] + DR	10	3 (30)	7 (70)	0	0	0
DL (*Nar*I) + DR	70	45 (64)	7 (10)	8 (11)	9 (13)	1 (1)
DL + DR SSalX (*Sal*I)	91	55 (60)	4 (4)	12 (13)	20 (22)	0

[a] Undigested (circular) or digested (enzyme shown in parenthesis) DL and DR were mixed and transfected into COS cells. Neo^R colonies were isolated and tested for their size by agarose gel electrophoresis.

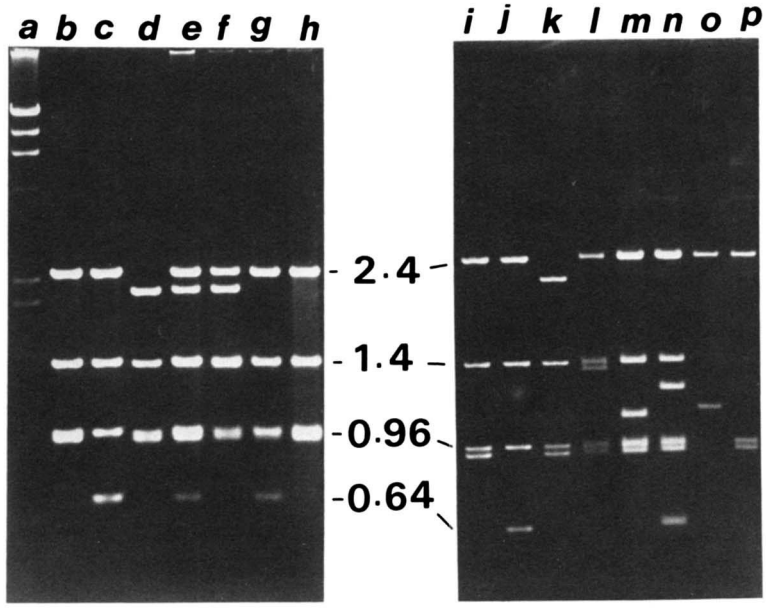

FIGURE 4.—Nature of plasmid DNA in neo^R colonies. Low MW DNA isolated from transfected COS cells was used to transform recombination deficient *E. coli*. Plasmid DNA was prepared from the neo^R colonies (MANIATIS, FRITSCH and SAMBROOK 1982), digested with *Pst*I and fractionated on agarose gels. Left panel, Unit size plasmids; right panel, smaller than unit size plasmids. Lane a, Lambda DNA digested with *Hin*dIII; lane b, pSV2neo; lane c, pSV2neo DR; lane d, pSV2neo DL; lane e, multimer containing a wild type, DL and DR; lane f, dimer containing a wild type and DL; lane g, dimer containing a wild type and DR; lane h, monomeric wild type; lane i, pSV2neo; lane j, pSV2neo DR; lane k, pSV2neo DL; lanes l–n, deleted dimers; lanes o–p, deleted monomers.

TABLE 4

Nature of neo^R monomeric molecules obtained from experiments with plasmids carrying polymorphic flanking markers

Substrates	No. of molecules (%)		
	*Hin*dIII-*Sma*I[a]	*Sma*I-*Xba*I[a]	*Sma*I-*Sma*I[a]
DL + DR SSalX	7 (26)	2 (7)	18 (67)
DL + DR SSalX (*Sal*I)	0 (0)	10 (24)	32 (76)

[a] Each of the column headings indicates the restriction enzyme sites that flank the neo gene in the recombinant monomers.

version of DR, whereas those containing two *Sma*I sites are either the result of reciprocal recombination or coconversion of DR and the adjoining *Xba*I site.

Nature of deletions: Several investigators have shown that autonomously replicating plasmids suffer deletions and mutations (CALOS, LEBKOWSKI and BOTCHAN 1983; RAZZAQUE, MIZUSAWA and SEIDMAN 1983; RAZZAQUE *et al.* 1984; LEBKOWSKI *et al.* 1984; ASHMAN and DAVIDSON 1984). Evidence to support the views that the deletions occur during the transfection procedure and dur-

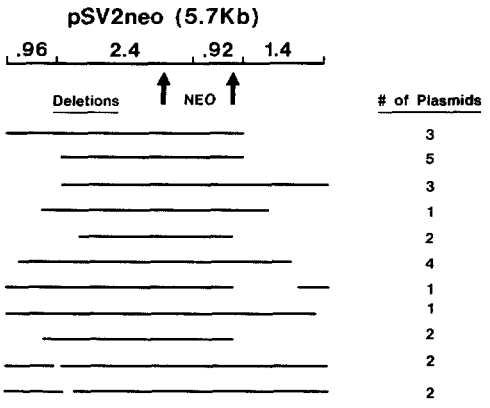

FIGURE 5.—Nature of deletions among neo^R plasmids. Deletions among plasmids, such as those shown in the right panel of Figure 4, are analyzed. Deletions within specific *Pst*I fragments were scored and indicated as spaces. The top line indicates the *Pst*I map of pSV2neo. Solid line indicates the region that was detected to be present in plasmids.

ing the plasmid replication was presented. We have tested the possibility that the generation of deletions is related to the recombination process. If this were the case, the deletions might be expected to be clustered around the neo gene. Neo^R plasmids were digested with *Pst*I, and the resulting pattern was compared with that obtained from a normal pSV2 neo plasmid. Results of this experiment are summarized in Figure 5. Digestion of wild-type molecules with *Pst*I yields four bands, 2.4, 1.4, 0.96 and 0.92 kb in length. Of these, the 2.4 and 0.92 kb bands carry the coding sequences for the neo gene. The disappearance of one or more of the four bands, to be replaced by bands of smaller size, indicates the site of deletion. There does not seem to be any specificity for the sites of deletion. These results indicate that the processes leading to deletions of the plasmids are not associated with the recombination process.

DISCUSSION

We have developed a novel method to study genetic aspects of recombination in mammalian cells. In the experimental system, we have utilized the fact that plasmids containing SV40 origin sequences are capable of autonomous replication in monkey cells transformed with origin-defective SV40 virus. A pair of plasmids, each of which carried a deletion in the selectable neo gene, were cotransfected, allowed to replicate and amplify, reisolated and used to transform *recA*$^-$ *E. coli* where we scored for recombinants by selection of the bacteria in neomycin. We obtained recombinants at a frequency of 3.9×10^{-3}. There are several lines of evidence that strongly support our view that the recombination has occurred in the COS cells, rather than in the bacteria. *RecA*$^-$ bacteria are known to have a severely reduced ability to mediate homologous recombination (CLARK and MARGULIES 1965). In fact, when the pSV2neo DL and DR plasmid DNAs were mixed and used to transform these bacteria, we obtained neo^R colonies at a frequency at least 100 times lower than that obtained with DNA passaged through COS cells. Since it is possible to argue that

passage through COS cells might have rendered the plasmids more amenable to recombination in bacteria, we have transfected COS cells with DL or DR alone. Low MW DNA isolated from these cells was mixed and used to transform bacteria. The frequency of neo^R colonies obtained from this experiment was also at least 100 times lower than that obtained from cotransfection. The most convincing evidence that recombination has occurred in the COS cells was obtained by directly examining the DNA obtained from these cells. When the DL/DR mixtures were passaged through COS cells, restriction fragments characteristic of the wild-type and doubly deleted plasmids were observed. All of these experiments clearly show that a majority, if not all, of the neo^R bacterial colonies we obtained are the result of transformation of bacteria with the wild-type plasmids generated by recombination in the mammalian cells.

Although the plasmids share homologies throughout their length, except in the regions of deletions, recombination has to occur in the interval between the 3′ end point of DL deletion and the 5′ end point of DR deletion to yield an intact neo gene. This length is 500 bp. Thus, the recombination frequency is approximately 8×10^{-3}/kb of homology.

Based on the observations that double-stranded breaks within the region of homology enhance homologous recombination between a chromosomal sequence and its counterpart introduced by DNA transfer methods (ORR-WEAVER, SZOSTAK and ROTHSTEIN 1981), and other observations, SZOSTAK *et al.* (1983) proposed a model of homologous recombination that is referred to as the double-strand break repair model. We have previously shown that similar double-strand breaks enhance homologous recombination between plasmids in human cells (KUCHERLAPATI *et al.* 1984b). We observed a similar increase of recombination in COS cells.

SZOSTAK *et al.* (1983) postulate that double-stranded breaks are key to the initiation of recombination, and the sites of such breaks are the sites of initiation of recombination. The results we have obtained are consistent with this hypothesis. We observed an increase in the frequency of neo^R colonies when one or both of the plasmids were cut within the neo gene region. Cutting outside the neo gene (*Eco*RI) did not have a similar effect. Although we did not obtain a high frequency of dimeric molecules, examination of these molecules provided useful information about the nature of recombinational events. When neither molecule was cut, the resulting dimers contained a wild-type gene and a DL, DR or doubly deleted gene. The DL or DR containing molecules clearly resulted from gene conversion, whereas those containing the doubly deleted gene most likely resulted from reciprocal recombination. The different products may reflect the site at which a double-strand break occurs. If it occurs at or near the deletion site it could result in gene conversion, and if it occurs at a site in between the end points of deletions, it may lead to reciprocal recombination. This view is substantiated by the fact that the molecules containing a double-strand cut always acted as recipients of genetic information (see Table 4). Our examination of the molecules generated by reactions mediated by cell extracts (KUCHERLAPATI, SPENCER and MOORE 1985) are also consistent with this view.

The monomeric molecules we have studied indicate that gene conversion is an important mechanism for the generation of wild-type molecules in the COS cell system. Having outside markers in the form of restriction enzyme site polymorphisms proved useful in this system. Gene conversion events can be unambiguously identified in those molecules that retained parental 5′ and 3′ markers, whereas other molecules (*i.e.*, those containing two *Sma*I sites) could have resulted from coconversion or reciprocal recombination. Additional markers are needed to clearly distinguish between these possibilities. Preliminary results obtained from the use of plasmids containing additional flanking markers support the view that gene conversion plays an important role in generating wild-type neo^R plasmids. Taken together, examination of the monomeric and dimeric molecules clearly indicates that reciprocal recombination and gene conversion are possible in COS cells.

The facts that (1) double-strand breaks at the sites of deletion enhance homologous recombination, (2) the sites of the break seem to be initiation points for recombination, (3) the molecule that is cut acts as a recipient of genetic information and (4) gap repair is an integral part of the recombination reaction support the double-strand break model of recombination proposed by SZOSTAK *et al.* (1983). LIN, SPERLE and STERNBERG (1984) proposed a model to explain some of their observations of intramolecular recombination. The results presented in this report are not explainable by this model; this may be because we have studied intermolecular reactions rather than intramolecular events.

A relatively high proportion of the plasmids we have obtained (20%) have suffered deletions. These deletions do not seem to have any relationship to the recombination process, because (1) similar deletions were observed among the amp^R colonies that represent the unrecombined population (results not shown) and (2) there is no site specificity for the deletions. The randomness of the deletions indicates that they should occasionally involve the neo gene rendering it nonfunctional. Thus, it is possible that the recombination frequencies that we obtain are underestimates. Since we select for neo^R, these deletions do not have any adverse effect on our ability to study the recombinational processes.

The COS cell system described here has many similarities to bacteriophage recombination in bacteria. The plasmids can replicate autonomously and undergo multiple rounds of recombination. A large number of recombinant products can be readily isolated and examined individually. This process is also analogous to the random spore analysis in yeast. The fact that the two products of recombination are occasionally retained in a single unit (dimers) makes it possible to deduce the precise nature of the recombination event. By using restriction enzyme sites as genetic markers, we can easily distinguish gene conversion events and reciprocal recombination events. Thus, the COS cell system promises to be an ideal system by which to study genetic aspects of recombination in mammalian cells, and we have begun to exploit it.

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LITERATURE CITED

ASHMAN, C. R. and R. L. DAVIDSON, 1984 High spontaneous mutation frequency in shuttle vector sequences recovered from mammalian cellular DNA. Mol. Cell. Biol. **4:** 2266–2272.

CALOS, M. P., J. S. LEBKOWSKI and M. R. BOTCHAN, 1983 High mutation frequency in DNA transfected into mammalian cells. Proc. Natl. Acad. Sci. USA **80:** 3015–3019.

CLARK, A. J. and A. D. MARGULIES, 1965 Isolation and characterization of recombination deficient mutants of *E. coli.* Proc. Natl. Acad. Sci. USA **53:** 451–459.

DESAINT VINCENT, B. R. and G. M. WAHL, 1983 Homologous recombination in mammalian cells mediates the formation of a functional gene from two overlapping gene fragments. Proc. Natl. Acad. Sci. USA **80:** 2002–2006.

FEINBERG, A. P. and B. VOGELSTEIN, 1983 High specific activity labeling of DNA restriction endonuclease fragments. Anal. Biochem. **132:** 6–13.

FOLGER, K. R., E. A. WONG, G. WAHL and M. R. CAPECCHI, 1982 Patterns of integration of DNA microinjected into cultured mammalian cells: evidence for homologous recombination between plasmid DNA molecules. Mol. Cell. Biol. **2:** 1372–1387.

GLUZMAN, Y., 1982 SV40 transformed simian cells support the replication of early SV40 mutants. Cell **23:** 175–182.

HIRT, B., 1967 Selective extraction of polyoma DNA from infected mouse cell culture. J. Mol. Biol. **36:** 365–369.

KUCHERLAPATI, R. S., D. AYARES, A. HANNAKEN, K. NOONAN, S. RAUTH, J. M. SPENCER, L. WALLACE and P. D. MOORE, 1984a Homologous recombination in monkey cells and human cell-free extracts. Cold Spring Harbor Symp. Quant. Biol. **49:** 191–197.

KUCHERLAPATI, R. S., E. M. EVES, K. Y. SONG, B. S. MORSE and O. SMITHIES, 1984b Homologous recombination between plasmids in mammalian cells can be enhanced by treatment of input DNA. Proc. Natl. Acad. Sci. USA **81:** 3153–3157.

KUCHERLAPATI, R. S., J. SPENCER and P. D. MOORE, 1985 Homologous recombination catalyzed by human cell extracts. Mol. Cell. Biol. **5:** 714–720.

LEBKOWSKI, J. S., R. B. DUBRIDGE, E. A. ANTELL, K. S. GREISEN and M. P. CALOS, 1984 Transfected DNA is mutated in monkey, mouse and human cells. Mol. Cell. Biol. **4:** 1951–1960.

LIN, F. W., K. SPERLE and N. STERNBERG, 1984 Model for homologous recombination during transfer of DNA into mouse L-cells: role for DNA ends in the recombination process. Mol. Cell. Biol. **4:** 1020–1034.

LOPATA, M. A., D. W. CLEVELAND and B. SOLLNER-WEBB, 1984 High level transient expression of a chloramphenicol acetyl transferase gene by DEAE-dextran mediated DNA transfection coupled with a dimethyl sulfoxide or glycerol shock treatment. Nucleic Acids Res. **12:** 5707–5717.

LOWY, D. R., E. RANDT and E. M. SCOLNICK, 1978 Helper independent transformation by unintegrated Harvey sarcoma virus DNA. J. Virol. **26:** 291–298.

MANDEL, M. and A. HIGA, 1970 Calcium dependent bacteriophage DNA infection. J. Mol. Biol. **53:** 159–162.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MILLER, C. L. and H. M. TEMIN, 1984 High efficiency ligation and recombination of DNA fragments by vertebrate cells. Science **220:** 606–609.

ORR-WEAVER, T. L., J. W. SZOSTAK and R. J. ROTHSTEIN, 1981 Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. USA **78:** 6354–6358.

RAZZAQUE, A., S. CHAKRABARTI, S. JOFFEE and M. SEIDMAN, 1984 Mutagenesis of a shuttle vector plasmid in mammalian cells. Mol. Cell. Biol. **4:** 435–441.

RAZZAQUE, A., H. MIZUSAWA and M. M. SEIDMAN, 1983 Rearrangement and mutagenesis of a shuttle vector plasmid after passage in mammalian cells. Proc. Natl. Acad. Sci. USA **80:** 3010–3014.

RUBNITZ, J. and S. SUBRAMANI, 1985 Rapid assay for extrachromosomal homologous recombination in monkey cells. Mol. Cell. Biol. **5:** 529–537.

SHAPIRA, G., J. L. STACHELEK, A. LETSOU, L. SOODAK and R. M. LISKAY, 1983 Novel use of synthetic oligonucleotide insertion mutants for the study of homologous recombination in mammalian cells. Proc. Natl. Acad. Sci. USA **80:** 4827–4831.

SMALL, J. and G. SCANGOS, 1983 Recombination during gene transfer into mouse cells can restore the function of deleted genes. Science **219:** 174–176.

SOUTHERN, E., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

SOUTHERN, P. J. and P. BERG, 1982 Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1:** 327–341.

SUSSMAN, D. J. and G. MILMAN, 1984 Short-term high-efficiency expression of transfected DNA. Mol. Cell. Biol. **4:** 1641–1643.

SZOSTAK, J. W., T. L. ORR-WEAVER, R. J. ROTHSTEIN and F. STAHL, 1983 The double strand break repair model for recombination. Cell **33:** 25–35.

UPCROFT, P., B. CARTER and C. KIDSON, 1980 Analysis of recombination in mammalian cells using SV40 genome segments having homologous overlapping termini. Nucleic Acids Res. **8:** 2725–2735.

VOLKERT, F. C. and C. S. H. YOUNG, 1983 The genetic analysis of recombination using adenovirus overlapping terminal DNA fragments. Virology **125:** 175–193.

WAKE, C. T. and J. H. WILSON, 1979 Simian virus 40 recombinants are produced at high frequency during infection with genetically mixed oligomeric DNA. Proc. Natl. Acad. Sci. USA **76:** 2876–2880.

WASMUTH, J. J. and L. VOCK HALL, 1984 Genetic demonstration of mitotic recombination in cultured Chinese hamster cell hybrids. Cell **36:** 697–707.

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