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Recombination and Deletion of Sequences in Shuttle Vector Plasmids in Mammalian Cells

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Shuttle vector plasmids were constructed with directly repeated sequences flanking a marker gene. African green monkey kidney (AGMK) cells were infected with the constructions, and after a period of replication, the progeny plasmids were recovered and introduced into bacteria. Those colonies with plasmids that had lost the marker gene were identified, and the individual plasmids were purified and characterized by restriction enzyme digestion. Recombination between the repeated elements generated a plasmid with a precise deletion and a characteristic restriction pattern, which distinguished the recombined molecules from those with other defects in the marker gene. Recombination among the following different sequences was measured in this assay: (i) the simian virus 40 origin and enhancer region, (ii) the AGMK *Alu* sequence, and (iii) a sequence from plasmid pBR322. Similar frequencies of recombination among these sequences were found. Recombination occurred more frequently in Cos1 cells than in CV1 cells. In these experiments, the plasmid population with defective marker genes consisted of the recombined molecules and of the spontaneous deletion-insertion mutants described earlier. The frequency of the latter class was unaffected by the presence of the option for recombination represented by the direct repeats. Both recombination and deletion-insertion mutagenesis were stimulated by double-strand cleavage between the repeated sequences and adjacent to the marker, and the frequency of the deletion-insertion mutants in this experiment was again independent of the presence of the direct repeats. We concluded that although recombination and deletion-insertion mutagenesis were both stimulated by double-strand cleavage, the molecules which underwent the two types of change were drawn from separate pools.

The rearrangement and recombination of DNA sequences in eucaryotic genomes have been linked to the activation of genes during development and oncogenesis. The consequences of these events may be visible at the level of the mitotic chromosome, appearing as sister chromatid exchanges, translocations, deletions, etc. These changes in chromosome structure reflect the interaction between chromosomal DNA and a variety of enzymatic functions in the cell nucleus. In recent years, many groups have assayed some of these functions by introducing appropriate viral or plasmid constructions into cells and by identifying and characterizing specific products which appear either extrachromosomally or integrated into the genome (2, 4, 7, 8, 13, 15, 16, 19, 22, 23, 25, 26, 28, 29, 31). An approach frequently used in studies of recombination between homologous sequences is to introduce plasmids carrying overlapping regions of genes arranged so that recombination between the repeated homologous sequences reconstructs the gene for which there is a selection. An alternative approach, and one we used here, is to flank a functional marker gene with repeats of any sequence (a coding potential is not required) and to monitor the recombination by the loss of the marker and by the production of a precise deletion.

Several groups have shown that appropriately placed double-strand breaks stimulate the frequency of inter- and intraplasmid recombination (13, 15). The importance of double-strand breaks as an initiating event for recombination has been emphasized in recent models of recombination pathways (15, 27), and it seems likely that recombination between homologous sequences on plasmids introduced into cells as superhelical templates is initiated by endonuclease-like activities in vivo (30).

Another assay for double-strand cleavage in vivo has been described recently by groups who used shuttle vector plasmids carrying bacterial marker genes (3, 6, 12, 14, 20, 21). After passage in mammalian cells, the plasmids are introduced into bacterial strains, and the integrity of the marker gene is determined by standard microbiological methods. It has been shown that plasmids with deletion mutations are generated in the mammalian cells (3, 6, 12, 14, 20, 21, 24) and that the frequency of these events is stimulated by appropriately placed double-strand breaks (20). Thus, cleavage events play an important role in both rearrangement and recombination of DNA in the transfection assays. The possibility of two quite different consequences after cleavage (deletion mutagenesis or recombination if homologous sequences are present) raises questions about the relative frequency with which the two products are formed and about the relationship between the two pathways. In the experiments described in this paper, we used shuttle vector plasmids which carry a marker gene flanked on either side with homologous sequences to ask whether the nature of the repeated sequences influences the frequency of recombination and whether the option for recombination influences the frequency of deletion mutagenesis after double-strand cleavage.

MATERIALS AND METHODS

Cells. AGMK cell lines CV1 and Cos1 (9) were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum and antibiotics. The bacterial strain used as a recipient for the shuttle vector plasmids was *Escherichia coli* HB101 (*recA13 hsdS20 galK2*).

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Enzymes. Restriction enzymes and other enzymes used for plasmid construction and analysis were from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) or International Biotechnologies, Inc. (New Haven, Conn.).

Construction of plasmids. The plasmids ppSC, pGSOE, and pAL11 are diagrammed in Fig. 1. All plasmids contained the simian virus 40 (SV40) early region fragment from the *Hpa*II site to the *Bam*HI site which contained the SV40 origin of replication and the gene for T antigen. In addition, they all contained the gene for the enzyme galactokinase (*galK*), which served as a marker. In plasmid ppSC, the *galK* gene was flanked by the 275-base-pair (bp) sequence from the *Bam*HI to the *Sal*I sites from plasmid pBR322. This sequence was arranged as a direct repeat. Plasmid pGSOE was constructed by inserting the 342-bp *Hin*dIII-*Pvu*II fragment from SV40 at the *Eco*RI site of plasmid pGS3 (21) so as to flank the *galK* gene with the origin and enhancer element of SV40 in a directly repeated arrangement. Plasmid pAL11 contained the 420-bp *Alu* sequence from AGMK cells (from M. Singer) (10) introduced in the *Eco*RI site and the *Bam*HI site so as to again produce a plasmid with the repeated element in the direct conformation.

Transfection, isolation, and characterization of mutants. Form I DNA was used in all experiments except as indicated. Cells were infected with 100 ng of plasmid by using DEAE-dextran as a facilitator (17). After 48 h, plasmids were harvested from monkey cells by the method of Hirt (11), purified, and treated with *Dpn*I to eliminate input DNA (18). The plasmids were then introduced to competent HB101 cells, and colonies carrying plasmids with defective *galK* genes were identified by colony color on MacConkey galactose agar plates or by resistance to 2-deoxygalactose (the plates contained 50 μg of ampicillin per ml) (1). Analysis of mutant plasmids by agarose gel electrophoresis, Southern blotting, and hybridization was done as described previously. In all experiments, sufficient colonies were screened to yield at least 100 mutants (5,000 to 10,000 colonies). For determination of recombination frequencies, at least 50 individual mutant plasmids were analyzed by restriction enzyme digestion.

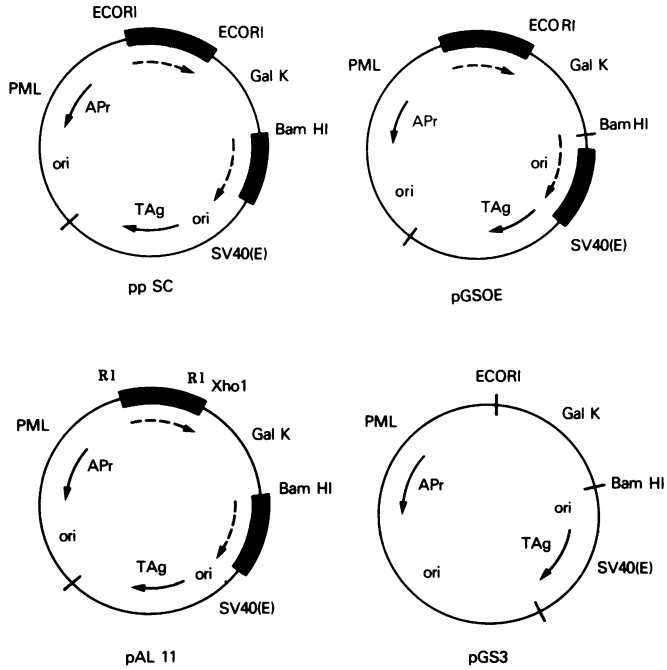

FIG. 1. Plasmids used in the recombination and mutagenesis assay. Plasmid ppSC contains two copies of the 275-bp *Bam*HI-*Sal*I fragment from plasmid pBR322 as direct repeats flanking the *galK* gene. Plasmid pGSOE contains a duplication of the 342-bp *Hin*dIII-*Pvu*II fragment from SV40 containing the origin and enhancer sequence. Plasmid pAL11 has the *Alu* sequence from AGMK cells as a direct repeat.

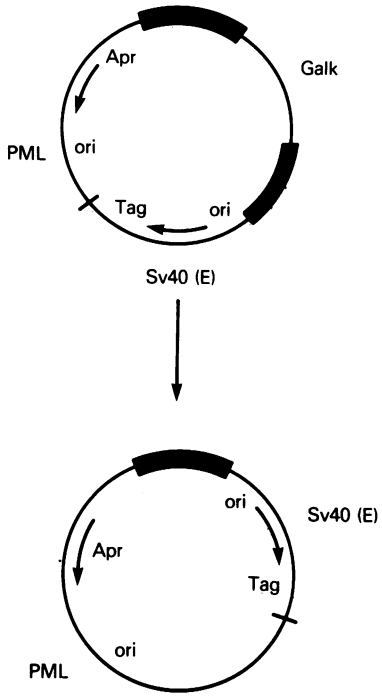

FIG. 2. Schematic diagram of the recombination between the direct repeated sequences, yielding a precisely deleted plasmid with the loss of the *galK* gene.

RESULTS

The plasmids used in these experiments are diagrammed in Fig. 1. The sequence from SV40 (the early region, origin, and enhancer elements) permitted replication in cells permissive for SV40 virus. The plasmids also contained information for replication and maintenance in bacteria. All the plasmids carried a bacterial gene which coded for the enzyme galactokinase, which served as a marker and could be readily assayed with standard microbiological procedures. Three of the constructions contained sequences present as direct repeats on either site of the *galK* gene. Recombination in the mammalian cells between the repeated sequences yielded a plasmid which had lost the *galK* gene and had one copy of the repeated sequence (Fig. 2). Bacteria carrying the recombined plasmid could be distinguished from those with the wild-type plasmid in a microbiological assay. The recombined plasmid had a deletion of precise size and a characteristic and diagnostic restriction pattern and thus could be distinguished from other plasmids which carry mutant *galK* genes and are also produced during passage in the mammalian cells (21). It is important to note that the bacterial strain (HB101) used in the screening of the plasmids after harvest from the mammalian cells was deficient in recombinational activity and that all the plasmids used in these experiments were stable in this host.

Recombination frequency of plasmids with different repeated sequences. Data from the first experiment are shown

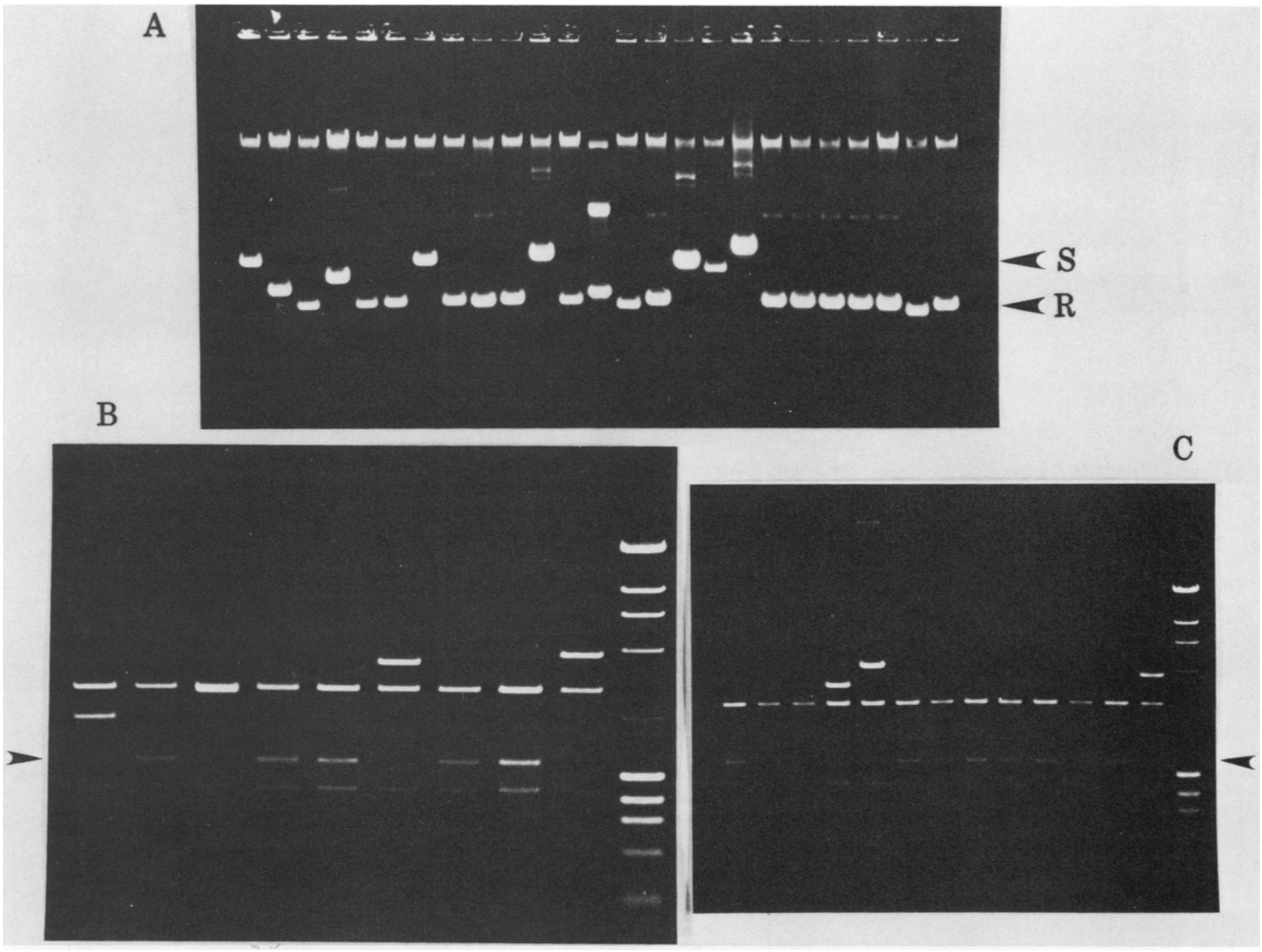

FIG. 3. Analysis of mutant plasmids and identification of recombined plasmids. (A) Cos1 cells were infected with plasmid ppSC. The mutant plasmids (*galK*$^-$) were picked, and the DNAs were electrophoresed in an agarose gel. The positions of the ppSC-sized plasmid (S) and the recombinants (R) are shown. (B and C) Restriction digestion analysis of the above-mentioned DNAs to identify the recombinants. The plasmid DNAs were cleaved with the restriction endonuclease *Bgl*I. For the recombinants, a fusion fragment of 1,494 bp (arrow) would be generated by the cleavage of the *Bgl*I site in the SV40 origin and in the ampicillin resistance (Ap^r) region of plasmid ppSC (Fig. 1). The molecular sizes of the bands were estimated relative to the migration of the standard DNA molecular weight markers (a mixture of *Hin*dIII fragments of λ DNA and *Hae*III fragments of φX174 DNA) shown in the extreme right lanes of panels B and C.

in Fig. 3. Plasmid ppSC contained as direct repeats the sequence from nucleotides 375 to 651 (*Bam*HI to *Sal*I) located in the tetracycline resistance region of plasmid pBR322. The SV40-transformed AGMK Cos1 cells were transfected with the plasmid. After 48 h, the progeny plasmids were harvested, purified, and introduced into HB101 cells. Colonies carrying plasmids which lacked a functional *galK* gene were identified, and the frequency of occurrence of these plasmids was determined. Plasmids were isolated from individual colonies and analyzed on agarose gels (Fig. 3A). In previous work, we found that spontaneous mutagenesis of plasmids occurs in the cells, and we anticipated that the population of mutant plasmids would contain some of these mutants in addition to the precise deletions generated by recombination. Inspection of the gel pattern suggested that molecules with the size expected from homologous recombination were present along with mutant plasmids as previously described (20, 21). A more definitive characterization was provided by digestion of the plasmids with *Bgl*I (Fig. 3B). A recombined plasmid with the predicted precise deletion had a *Bgl*I fragment of 1,495 bp which was the result of the fusion of sequences on either side of those lost through recombination. We confirmed the colinearity of these flanking sequences in the fusion fragment by hybridization with the appropriate fragments as probes to the *Bgl*I digests of the mutant plasmids (data not shown). We found that the restriction digestion patterns of the mutant plasmids accurately identified those which arose through recombination. By using these analytical procedures, we found that the frequency of mutant plasmids after passage of plasmid ppSC in Cos1 cells was 2%, whereas the frequency of recombined plasmids was 1%. The experiment was repeated in CV1 cells, and we found, reproducibly, an overall mutation frequency of 1.5% and a recombination frequency of 0.3%. Thus, the CV1 cells appeared to be as active as the Cos1 cells in generating mutant plasmids and a fewfold less active than Cos1 in producing recombined plasmids.

We then repeated the experiments with the construction in which the origin and enhancer regions of SV40 were repeated (plasmid pGSOE). We were curious whether the

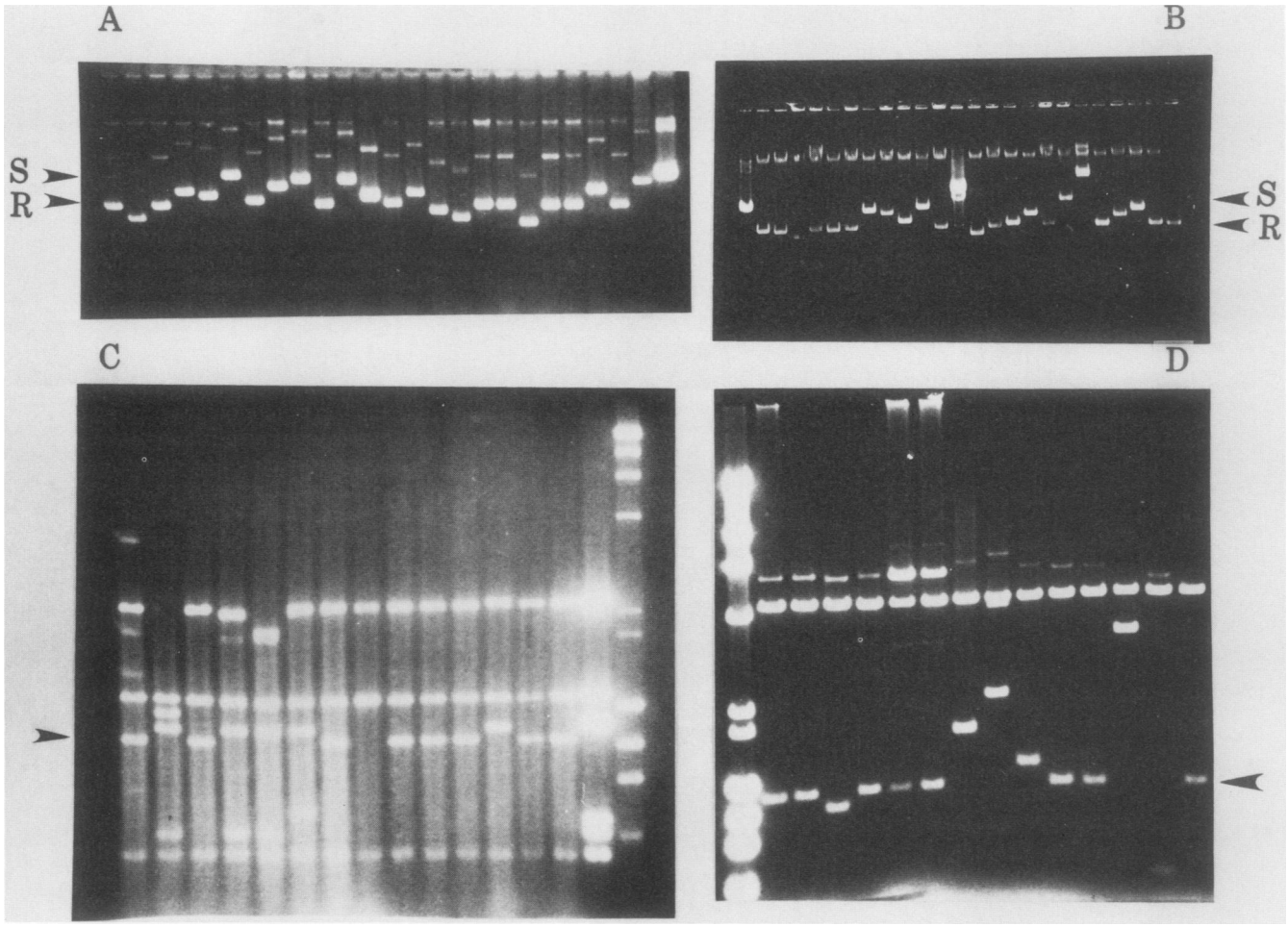

FIG. 4. Analysis of mutant plasmids derived from the pGSOE and pAL11 plasmids in Cos1 cells. (A and B) Mutant plasmids pGSOE (A) and pAL11 (B) were electrophoresed in an agarose gel. The positions of starting plasmids (S) and recombinants (R) are shown. (C) Diagnostic restriction digestion of pGSOE mutants with *Taq*I. As shown above (Fig. 3), a fusion fragment of 1,175 bp (arrow) is produced by the *Taq*I sites present in the ampicillin resistance and SV40 T antigen regions of the recombinants. (D) Diagnostic restriction digestion of pAL11 mutants with *Sca*I and *Kpn*I. The predicted fusion fragment for the recombinants is 1,263 bp (arrow) which arose from cleavage at the unique *Sca*I and *Kpn*I sites present in the ampicillin resistance and SV40 early regions, respectively, of pAL11 mutants. The standard DNA molecular weight markers were run with the samples as in the legend to Fig. 3.

presence of repeated sequences with the potential for alternate DNA conformations, such as the Z form and cruciforms, might affect the recombination frequency. Furthermore, the Cos1 cells constitutively produce a functional T antigen protein which interacts with some of the sequences in the repeated elements in this construction. However, we found that the results were very similar to those obtained with plasmid ppSC (Fig. 4A and C). The mutation frequency of this plasmid after passage through Cos1 cells was 3%, and the recombination frequency was again 1%. With CV1 cells we found the mutation frequency to be 1.3%, whereas the recombination frequency was 0.2%, fivefold lower than in the Cos1 cells.

The last plasmid used in these experiments, pAL11, had as direct repeats the *Alu* sequence from AGMK cells. This sequence was of interest because of its high copy number and its interspersion in the genome such that the *Alu* elements are often present as direct repeats separated by single copy DNA (10). We wondered if the stability of this arrangement in the genome would be reflected in the frequency of recombination between the *Alu* repeats in the transfected plasmid. However, we again found that the recombination frequency in both Cos1 and CV1 cells was similar to that with the other two constructions (Fig. 4B and D). The results of these experiments are summarized in Table 1.

Is there a relationship between recombined and deletion mutant plasmids? Also shown in Table 1 are the spontaneous mutation frequencies in the two cell lines for plasmid pGS3 (20). This plasmid contains the *galK* gene, but there are no repeated sequences. Although homologous recombination cannot be assayed with this plasmid, it does report the spontaneous mutagenizing activities present in the two cell lines. We described the types of mutant plasmids recovered after passage in these cells and concluded that the deletion-insertion mutants are related to cleavage of the plasmid in vivo by endogenous endonucleaselike activities (20). Consistent with this conclusion is our observation that the frequency of deletion mutant plasmids is enhanced 10-fold after infection of the mammalian cells with plasmids linearized by cleavage at a site adjacent to the *galK* gene (20). Similarly, it has been shown by several groups that appro-

TABLE 1. Recombination frequency of plasmids with different repeated sequences[a]

Plasmid	Cell type	Mutation frequency (%)	Recombination frequency (%)
ppSC	CV1	1.5	0.3
	Cos1	2	1
pGSOE	CV1	1.3	0.2
	Cos1	3	1
pAL11	CV1	1.5	0.4
	Cos1	3	1.2
pGS3	CV1	1.1	
	Cos1	1.2	

[a] CV1 or Cos1 cells were transfected with the plasmids (100 ng/150-cm^2 petri dish) as indicated. After 48 h, the progeny plasmids were recovered, and the frequency of plasmids with defective *galK* genes was determined. Mutant plasmids were isolated, and those with the precise deletion arising from recombination were identified by restriction digestion.

priately placed, double-strand breaks can greatly stimulate the frequency of recombination between homologous sequences in transfected DNA (13, 15). Analysis of the data presented in the agarose gel profiles in Fig. 3 and 4 and in Table 1 indicated that both recombined and deletion mutant plasmids appeared in the mutant population and, furthermore, that the recombined plasmids appeared in addition to the mutant plasmids which would be seen with the plasmid which lacked the recombination option. Although the available evidence indicated that the production of both classes of molecules was stimulated by a double-strand cleavage, these data would suggest that the two types of *galK*-defective plasmids were derived from separate pools of precursor molecules.

Recombination and deletion mutagenesis were stimulated by a double-strand cleavage. We addressed this possibility in an experiment in which cells were transfected with the linearized form (by cleavage with *Xho*I; Fig. 1) of one of the plasmids with direct repeats (pAL11). Cleavage with *Xho*I produced a linear molecule with the *Alu* sequence now present as a terminal duplication. In a companion preparation, the same plasmid was cleaved with *Eco*RI, which released one copy of the *Alu* sequence and thus produced a linear molecule with no repeated elements. Cos1 cells were transfected with the two DNA preparations, and the experiment was performed as before. The agarose gel profile of some of the plasmids with a defective *galK* gene from the infection with the linearized plasmid with the terminal duplication is shown in Fig. 5. With the criteria described previously, we found the total mutation frequency to be 26% when cleaved with *Xho*I, whereas the recombination frequency was 16%. The nonrecombined mutant plasmids were deletion mutants as was the case with the mutants recovered from the infections with the linear plasmid without the terminal duplication (with a mutation frequency of 11% when cleaved with *Eco*RI). When the deletion mutant plasmids from both infections were examined by restriction enzyme digestion, it was found that the majority (85 to 90%) had lost fragments containing and adjacent to the site of cleavage (data not shown; see reference 20). The results of this experiment confirmed the stimulatory effect of double-strand cleavage in the generation of the deletion mutant and of the recombined plasmids and supported the conclusion that the two classes of molecules were formed independently of one another.

DISCUSSION

Most studies of recombination in mammalian cells use segments of selectable genes as participants in the recombination reactions. The product of the reaction is a reconstructed gene which codes for an activity which can be assayed or for which there is a straightforward selection (4, 13, 15, 19, 22, 25, 28, 31). This protocol has obvious advantages but imposes certain limitations on the nature of the sequence involved in the recombination events. The experimental design used here permitted any sequence of interest to be the target for the recombination process. (A variation of this approach has been used recently to study site-specific recombination of lymphoid cells [5].) We used this approach to measure the frequency of recombination of AGMK cells among three different types of sequences. They were (i) the SV40 origin and enhancer region with a variety of alternate conformational possibilities (cruciform and Z DNA), (ii) the *Alu* sequence from AGMK cells, which is a stable repeated element in the mammalian genome, and (iii) a sequence from plasmid pBR322 with limited alternate

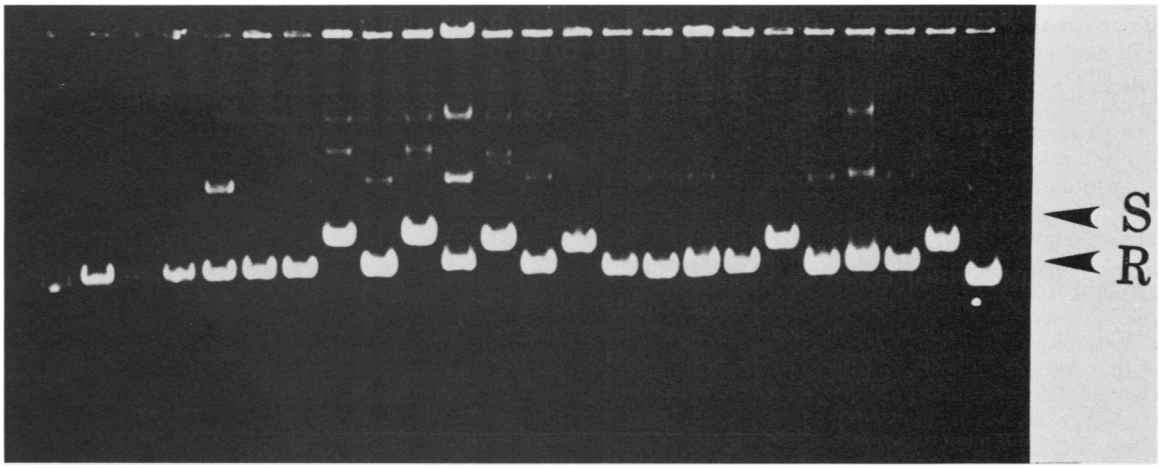

FIG. 5. Agarose gel electrophoresis of the DNAs from the mutant plasmids derived from the transfection of plasmid pAL11 linearized with *Xho*I (Fig. 1) in Cos1 cells. The positions of the starting plasmid (S) and recombinants (R) are indicated.

structural possibilities. We found that these three sequences gave very similar results in this recombination assay. The recombined molecules appeared in the progeny plasmid population at similar frequencies with all constructions. We analyzed the recombined plasmids by fine restriction analysis and found in all cases that the repeated elements remaining on the plasmid were indistinguishable from the starting sequences. Despite the presence of duplicated sequences in both the SV40 origin-enhancer segment and the *Alu* sequence, we found no evidence of staggered crossover events.

The metabolism of free ends of DNA is receiving increasing attention as their importance in recombination and rearrangement events has become apparent (32). We have used one of our constructions to address questions about the fate of free ends in the AGMK cells. The design of the plasmid permitted the measurement of the frequency of several different products which were the results of the several different options faced by free ends in the cells. These included recircularization of the plasmid with no effect on the marker gene, recircularization after events which led to deletions in the marker gene, and recircularization after recombination between the repeated sequences. Note that the position of the cleavage site in plasmid pAL11 (the *Xho*I site; Fig. 1) is adjacent to and outside the marker. The choice of the site eliminated the production of those small deletion mutants which would arise from "nibbling" of the ends (30). Linearization of the pAL11 plasmid before transfection produced substantial enhancement of the frequency of both recombined and deletion mutant plasmids in the progeny plasmid population. The results are in agreement with those of previous studies in which one or the other type of product was assayed (13, 15, 20). The interesting feature of our experiments was that the increase in frequency of the deletion mutants was independent of the presence of the terminally duplicated sequence. We interpreted these results as indicating that the pool of molecules which serve as substrates for the recombination process is distinct from that pool which supplies the deletion mutants, although both processes are initiated by a double-strand break. Two current models for the mechanism of recombination invoke double-strand cleavage and the generation of free ends as the initiating and rate-limiting step in the recombination pathway (15, 27). Although there are significant differences between the two models, both propose that exonucleases then expose single-strand regions which can then become involved in pairing with homologous sequences. In neither model is the process dependent on the recognition of sequence homology before the action of the endo- and exonucleases. The implication of these models is that if there are no duplications of a particular sequence then in the simplest case, molecules on this pathway would be degraded. In other words, as proposed by J. Wilson (personal communication), the presence of the repeats actually saves at least some fraction of these molecules from degradation, presumably by providing the option for a primer-template hybrid for gap repair synthesis. Our observation that the presence or absence of the potential for recombination had no effect on the frequency of deletion events suggested that intermediates formed on the recombination pathway were not converted to deletion mutants. Thus, if, as suggested, linear molecules with single-strand ends were formed, these did not contribute appreciably to the deletion mutants. The simplest explanation for the generation of deletion mutants is that they were the consequence of a second endonucleolytic cleavage or a double-strand exonuclease activity after an initial cleavage. Recircularization would prevent further degradation.

Experiments with plasmids microinjected into nuclei indicate that recombination is a nuclear event (8). As noted by several authors, the frequency of recombination and deletion mutagenesis is significantly higher for DNA in transfections than in the genome. Apparently, the transfected DNA is much more sensitive to the relevant nuclear factors than genomic sequences. We and others have suggested that the chromatin structure (or lack of it) of the DNA in the transfection may distinguish it from chromosomal DNA (20, 21). It remains to be seen whether these differences are simply quantitative or whether the actual mechanisms of recombination and rearrangement are also different.

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LITERATURE CITED

1. **Alper, M. D., and B. N. Ames.** 1975. Positive selection of mutants with deletions of the *gal-chl* region of the *Salmonella* chromosome as a screening procedure for mutagens that cause deletions. J. Bacteriol. **121**:259–266.
2. **Anderson, R. A., T. Kakauer, and R. D. Camerini-Otero.** 1982. DNA mediated gene transfer: recombination between cotransferred DNA sequences and recovery of recombinants in a plasmid. Proc. Natl. Acad. Sci. USA **79**:2748–2752.
3. **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.
4. **Bandyopadhyay, P., S. Watanabe, and H. M. Temin.** 1984. Recombination of transfected DNAs in vertebrate cells in culture. Proc. Natl. Acad. Sci. USA **81**:3476–3480.
5. **Blackwell, T. K., and F. W. Alt.** 1984. Site specific recombination between immunoglobulin D and JH segments that were introduced into the genome of a murine pre B cell line. Cell **37**:105–112.
6. **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.
7. **Folger, K. R., K. Thomas, and M. R. Capecchi.** 1985. Nonreciprocal exchanges of information between DNA duplexes coinjected into mammalian cell nuclei. Mol. Cell. Biol. **5**:59–69.
8. **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 injected plasmid DNA molecules. Mol. Cell. Biol. **2**:1372–1387.
9. **Gluzman, Y.** 1981. SV40 transformed simian cells support the replication of early SV40 mutants. Cell **73**:175–182.
10. **Grimaldi, G., C. Queen, and M. F. Singer.** 1981. Interspersed repeated sequences in the African green monkey genome that are homologous to the human *Alu* family. Nucleic Acids Res. **9**:5553–5568.
11. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
12. **Kretschmer, P. J., A. H. Bowman, M. H. Huberman, L. Sanders-Haigh, L. Killos, and W. F. Anderson.** 1981. Recovery of recombinant bacterial plasmids from *E. coli* transformed with DNA from microinjected mouse cells. Nucleic Acids Res. **9**:6199–6216.
13. **Kucherlapati, R. S., E. M. Eves, K. Y. Song, B. S. Morse, and O. Smithies.** 1984. Homologous recombination between plasmids in mammalian cells can be enhanced by treatment of input DNA. Proc. Natl. Acad. Sci. USA **81**:3153–3157.
14. **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.

15. **Lin, F.-L., 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.
16. **Lin, F.-L., and N. Sternberg.** 1984. Homologous recombination between overlapping thymidine kinase gene fragments stably inserted into a mouse cell genome. Mol. Cell. Biol. **4**:852–861.
17. **McCutchan, J. H., and J. S. Pagano.** 1968. Enhancement of the infectivity of simian virus 40 deoxyribonucleic acid with diethyl amino ethyl dextran. J. Natl. Cancer Inst. **41**:351–356.
18. **Peden, K. W. C., J. M. Pipas, S. Pearson-White, and D. Nathans.** 1980. Isolation of mutants of an animal virus in bacteria. Science **203**:1392–1396.
19. **Pomerantz, B. J., M. Naujokas, and J. A. Hassell.** 1983. Homologous recombination between transfected DNAs. Mol. Cell. Biol. **3**:1680–1685.
20. **Razzaque, A., S. Chakrabarti, S. Joffe, and M. Seidman.** 1984. Mutagenesis of a shuttle vector plasmid in mammalian cells. Mol. Cell. Biol. **4**:435–441.
21. **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.
22. **Robert de Saint Vincent, B., and G. M. Wahl.** 1983. Homologous recombination in mammalian cells mediates formation of a functional gene from two overlapping gene fragments. Proc. Natl. Acad. Sci. USA **80**:2002–2006.
23. **Rubnitz, J., and S. Subramani.** 1984. The minimum amount of homology required for homologous recombination in mammalian cells. Mol. Cell. Biol. **4**:2253–2258.
24. **Shenk, T. E., J. Carbon, and P. Berg.** 1976. Construction and analysis of viable deletion mutants of simian virus 40. J. Virol. **18**:664–671.
25. **Small, T., and G. Scangos.** 1983. Recombination during gene transfer into mouse cells can restore the function of deleted genes. Science **219**:174–176.
26. **Subramani, S., and P. Berg.** 1983. Homologous and nonhomologous recombination in monkey cells. Mol. Cell. Biol. **3**:1040–1052.
27. **Szostak, J. W., T. L. Orr-Weaver, R. J. Rothstein, and F. W. Stahl.** The double-strand break repair model for recombination. Cell **33**:25–35.
28. **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**:2725–2736.
29. **Upcroft, P., B. Carter, and C. Kidson.** 1980. Mammalian cell functions mediating recombination of genetic elements. Nucleic Acids Res. **8**:5835–5844.
30. **Wake, C. T., T. Gudewicz, T. Porter, A. White, and J. H. Wilson.** 1984. How damaged is the biologically active subpopulation of transfected DNA? Mol. Cell. Biol. **4**:387–398.
31. **Wake, C. T., and J. H. Wilson.** 1980. Defined oligomeric SV40 DNA: a sensitive probe of general recombination in somatic cells. Cell **21**:141–148.
32. **Wilson, J. H., P. B. Berget, and J. M. Pipas.** 1982. Somatic cells efficiently join unrelated DNA segments end-to-end. Mol. Cell. Biol. **2**:1258–1269.