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Recombination Hotspot Activity of Hypervariable Minisatellite DNA Requires Minisatellite DNA Binding Proteins

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Abstract

Hypervariable minisatellite DNA repeats are found at tens of thousands of loci in the mammalian genome. These sequences stimulate homologous recombination in mammalian cells [Cell **60**:95–103]. To test the hypothesis that protein-DNA interaction is required for hotspot function *in vivo*, we determined whether a second protein binding nearby could abolish hotspot activity. Intermolecular recombination between pairs of plasmid substrates was measured in the presence or absence of the *cis*-acting recombination hotspot and in the presence or absence of the second *trans*-acting DNA binding protein. Minisatellite DNA had hotspot activity in two cell lines, but lacked hotspot activity in two closely related cell lines expressing a site-specific helicase that bound to DNA adjacent to the hotspot. Suppression of hotspot function occurred for both replicating and non-replicating recombination substrates. These results indicate that hotspot activity *in vivo* requires site occupancy by minisatellite DNA binding proteins.

INTRODUCTION

Homologous recombination is the exchange of genetic information between DNA molecules with sequence homology. Although the amount of recombination that occurs between two markers is generally a function of the intermarker physical distance, this is not strictly the case. Certain regions of chromosomes, called “recombination hotspots,” promote a high rate of recombination in their vicinity. Recombination hotspots may contribute a significant fraction of all recombination that occurs in the genome (2–5).

Recombination hotspots were first described in bacteriophages (6) and have since been found in a variety of organisms including humans (3–5). Presumably, discrete proteins interact with hotspots to mediate their biological activity. In *Escherichia coli* the multifunctional RecBCD enzyme interacts with the 8 bp Chi sequence to stimulate recombination and many of the molecular steps of this pathway have been revealed (7–10). Both *cis*- and *trans*-acting components of fungal hotspots have been either directly demonstrated or inferred from genetic studies (11–21). For example, binding of the Mts1/Mts2 heterodimeric protein (22) to the 7 bp M26 site (15) in the fission yeast *Schizosaccharomyces pombe* activates a meiosis-specific hotspot (21). In mammals *cis*- and *trans*-acting hotspot components have also been inferred genetically. For example, several recombination hotspots have been mapped within the mouse major histocompatibility complex (MHC) (23–33). However, the discrete DNA sites responsible for hotspot activity within metazoan chromosomes have not been identified.

Hypervariable minisatellite DNA is composed of short, dG·dC-rich core sequences, usually 10 to 20 bp long, that are repeated in tandem arrays at more than 15,000 loci in the

mammalian genome (34–39). Considerable circumstantial evidence supports a role for minisatellite DNA in promoting recombination in vivo (5). The evidence includes a high rate of meiotic change at minisatellite loci (40), localization of chiasmata at minisatellite DNA within recombining chromosomes (41), and the finding of these sequences at some hotspots of the mouse MHC (27). Activity of the Lmp2 MHC hotspot requires significant homology between recombining chromosomes, with the number of copies of the minisatellite motif being most important (42), further suggesting that minisatellite DNA is necessary component of hotspot function.

The development of plasmid-based assays for recombination in somatically dividing mammalian cells in culture has become a useful tool because the basic biochemical process of interplasmid recombination are the same as those that occur within chromosomes (43, 44). We and others have used such systems to determine whether specific DNA sequences can function as recombination hotspots (45–50). Six copies of a hypervariable minisatellite DNA core sequence (SAT: 5'-AGAGGTGGGCAGGTGG-3') functioned as a hotspot in human cells and promoted recombination within a distal test interval (49).

A number of hypervariable minisatellite DNA binding proteins have been detected in whole-cell and nuclear extracts of from variety of organisms, including humans (50–55). However, little is known about the biochemistry of these factors and no DNA or protein sequence data have been reported.

In this study we tested the hypothesis that minisatellite DNA binding proteins are required for the hotspot activity of minisatellite DNA in vivo. To do so we determined whether binding of a site-specific DNA helicase (SV40 T antigen) adjacent to the minisatellite DNA could competitively inhibit its hotspot function. Our results indicate that hotspot activity requires site occupancy by minisatellite DNA binding proteins.

MATERIALS AND METHODS

Cell Lines

The human bladder carcinoma cell line EJ, simian CV-1 cells, CV-1 derived COS-1 cells expressing SV40 virus large T antigen, and human CCD156 cells expressing mutant SV40 large T antigen were grown as adherent cultures. Cells were cultured at 37°C in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, in a 7% CO₂ atmosphere.

Recombination Substrates

The recombination substrates were derived from the prokaryotic-eukaryotic shuttle vector pSV2neo (56), which can replicate in *E. coli* and in mammalian cells expressing the SV40 large T antigen. The neomycin phosphotransferase (*neo*) gene from transposon Tn5 is flanked by the SV40 early promoter and poly(A) addition signals. This gene confers resistance to kanamycin and neomycin in bacteria and to the aminoglycoside analog G418 in mammalian cells. Two deletion plasmids, designated DL (deletion left) and DR (deletion right), were constructed by removing the 248 bp *NarI-NarI* fragment and the 283 bp *NaeI-NaeI* fragments, respectively (57). There are 501 bp of homology between the sites of the two deletions within which recombination can occur to give rise to a wild-type *neo* gene and confer resistance to kanamycin (in *E. coli*) or to G418 (in mammalian cells). The substrates used were pLCKS (DL) and pDR-D86 (DR), which contain silent, modified restriction sites to permit product analyses (48, 58, 59).

Six copies of the hypervariable minisatellite DNA sequence (SAT₆) were cloned between the *StuI* and *HindIII* sites of pSV2neo and the two deletion plasmids pLCKS (DL) and pDR-

D86 (DR) to generate the recombination substrates pDLSAT and pDRSAT, respectively. This placed the minisatellite DNA outside of the region within which recombination must occur, 237 bp from the site of the nearest deletion.

Transfections and Transformations

All DNAs used for transfections were purified by cesium chloride density gradient centrifugation followed by extensive dialysis against 10 mM Tris hydrochloride, 1 mM EDTA, pH 7.4 (TE).

Transfection of EJ, CV-1, and CCD-156 cell lines was by a modified calcium phosphate precipitation method of Lowy et al. (60) as previously described (48, 49). Transfected cells were incubated for 24 h in the absence of selection and then transferred to 100 mm plates for selection in culture medium containing G418 at 400 µg/ml. G418^r colonies were visible at 7 to 10 days and were stained with Giemsa after two wks of selection.

DNA was introduced into COS-1 cells by the DEAE-dextran method (61, 62) as previously described (59). Cells were cultured in the absence of selection for 48 h prior to the isolation of low molecular weight episomal DNA by the procedure of Hirt (59, 63).

Low molecular weight DNA isolated from COS-1 cells was introduced into the *recA* mutant *E. coli* strain DH-5 by CaCl₂ mediated transformation (64).

Replication Assay

The established replication assay is based upon the conversion of *DpnI*-sensitive, adenine-methylated plasmid DNA from *dam*⁺ *E. coli* into *DpnI*-resistant DNA upon replication in mammalian cells, which do not methylate adenine at the *DpnI* site (59, 65–69). COS-1 cells were cotransfected with 100 ng of experimental plasmid and 50 ng of internal replication control plasmid p2X21ori. After 56 h of incubation in the absence of selection low molecular weight DNA was prepared (59, 63). The DNA was digested to completion with a 3-fold excess of *SalI*, which cuts only once within each plasmid, and with a 15-fold excess of *DpnI*, which cleaves unreplicated DNA into multiple fragments. The digested DNA was analyzed by Southern blotting (70) using random hexanuclotide primed pBR322 DNA as a probe. Autoradiographs were prepared with Kodak XAR-5 film and the relevant band intensities were quantified using an LKB UltraScan XL laser densitometer. The replication efficiencies of the experimental plasmids were determined relative to the total replication efficiencies of experimental plasmids and the internal replication control plasmid, p2X21ori.

RESULTS

Experimental Strategy

The pSV2neo shuttle vector confers resistance to ampicillin in bacteria. It also confers resistance to kanamycin or neomycin in bacteria and to G418 in mammalian cells (56) (Fig. 1). The two pSV2neo deletion plasmids, DL and DR, are derivatives that contain deletions within the 5' and 3' regions of the *neo* gene, respectively (Fig. 1). These deletion mutations are nonreverting and noncomplementing, so the DL and DR plasmids are incapable of conferring resistance to kanamycin or G418. However, in some of the cells cotransfected with DL and DR a homologous recombination event, within the interval between the two deletions, gives rise to a wild-type *neo* gene (Fig. 2). It is therefore possible to select directly for recombinant plasmid molecules in both mammalian cells and in *E. coli*. This enabled us to use the exact same sets of substrates to compare rates of recombination between replicating and nonreplicating substrates, with or without the *cis*-acting hypervariable

minisatellite (SAT₆) DNA sequence, and in the presence or absence of the SV40 large T antigen (Fig. 1).

Lack of Recombination Hotspot Activity in COS-1 Cells

To determine whether hypervariable minisatellite DNA acted as a recombination hotspot in the presence of SV40 T antigen, we introduced the recombination substrates into COS-1 cells, allowed the plasmids to replicate for 48 h, harvested low molecular weight DNA, and transformed recombination deficient *E. coli* bacteria. Selection for ampicillin resistance revealed the total plasmid titer and selection for kanamycin resistance revealed the recombinant titer.

When the DL and DR plasmids were introduced separately into COS-1 cells and the resulting low molecular weight DNA was cotransformed into *E. coli*, no kanamycin resistant recombinants were obtained (Table 1). Identical results were obtained with the DLSAT and DRSAT substrates (Table 1). This demonstrates that the *recA* mutant *E. coli* are incapable of recombining these plasmid substrates, even after they have been passed through mammalian cells.

When the DL and DR plasmids were introduced together into COS-1 cells and subsequently introduced into *E. coli*, a recombinant frequency of 9.0×10^{-3} was obtained (Table 1). We conclude that COS-1 cells are proficient at catalyzing recombination between the deletion plasmids. Intriguingly, cotransfection of DLSAT and DRSAT plasmids produced a recombinant frequency of 6.4×10^{-3} , indicating that the minisatellite DNA was not a functional hotspot within COS-1 cells. This was not an intrinsic property of the substrates, because these same DLSAT and DRSAT plasmids exhibit hotspot activity (10- to 40-fold the recombinant frequency obtained from DL $\times$ DR crosses) when used as nonreplicating recombination substrates in a variety of mammalian cell types (49) (W.P.W., unpublished observations). Thus, some feature of COS-1 cells, either the T antigen binding or substrate replication, eliminated the hotspot function of the hypervariable minisatellite DNA.

DLSAT and DRSAT Replicate with Lower Efficiency than DL and DR in COS-1 Cells

The minisatellite DNA was cloned between the *StuI* and *HindIII* sites of pSV2neo to place it into close proximity to the T antigen binding site (Fig. 1). This cloning strategy might have altered the replication efficiencies of the respective substrates. As shown in Fig. 3b, introduction of the minisatellite DNA mutated the last nucleotide of the T antigen binding site. Plasmids containing similar mutations are capable of T antigen-dependent replication, but at a reduced level (71). If this alteration reduced the replication efficiencies of DLSAT and DRSAT, and the amount of replication or the substrate concentrations contributed to the recombinant frequencies, hotspot activity of the minisatellite DNA might have been present, but obscured due to differences in the replication efficiencies of the substrates.

We used an established procedure (59, 61, 62, 67, 68) to determine the relative replication efficiencies of the various recombination substrates. COS-1 cells were cotransfected with the individual recombination substrates and an internal replication control plasmid, p2X21ori, which contains two copies of the SV40 origin of replication and replicates with high efficiency in COS-1 cells (68). After a period of culture, low molecular weight DNA was purified and digested with two restriction enzymes. *SalI* was used to linearize the plasmids in the presence of a large excess of *DpnI*. Because the input plasmids had been generated in *dam*⁺ *E. coli*, they were adenine methylated and were cleaved into numerous small fragments by *DpnI* digestion, leaving intact only those plasmid molecules that had undergone at least one round of DNA replication in the COS-1 cells.

Southern blotting revealed that the pX21ori and pSV2neo deletion plasmids had all replicated in the COS-1 cells (Fig. 3a). However, the replication efficiencies of DLSAT and DRSAT were approximately an order of magnitude lower than those of DL and DR (Fig. 3b). Therefore, the frequency of kanamycin resistant colonies may not accurately reveal the effect of the minisatellite DNA upon recombination between the replicating substrates because the effective substrate concentration in the DLSAT $\times$ DRSAT cross was lower than that in the DL $\times$ DR cross.

Hypervariable Minisatellite DNA fails to Stimulate Recombination Between Substrates with Equivalent Replication Efficiencies

In order to accurately measure the effect of the minisatellite DNA upon recombination in COS-1 cells, it was first necessary to adjust the replication efficiencies of the substrates to an equivalent level. This was accomplished by removing the *StuI-HindIII* fragment from DL and the *StuI-SmaI* fragment from DR (59). The resulting modified DL and DR molecules, DL Δ SH and DR Δ SS, each had single nucleotide mutations in the last position of the T antigen binding site, like those changes introduced in constructing the DLSAT and DRSAT substrates (Fig. 3b). The substrates were subject to the replication assay and found to be capable of replication, but at a reduced level (Fig. 3a). The replication efficiencies of DL Δ SH, DR Δ SS, DLSAT, and DRSAT were essentially equivalent (Fig. 3b).

Once the replication efficiencies had been equalized, we cotransfected COS-1 cells with various combinations of the deletion plasmids and measured the recombinant frequencies. The DL Δ SH $\times$ DR Δ SS cross produced a recombinant frequency of 7.4×10^{-3} , which is very close to frequency of recombinants (9.0×10^{-3}) produced from the DL $\times$ DR cross (Table 1). We conclude that the recombinant frequency is largely independent of the substrate concentration, at least for the range of replication efficiencies tested. Furthermore, each of the crosses containing DLSAT, DRSAT, or both DLSAT and DRSAT produced recombinant frequencies similar to those of the DL Δ SH $\times$ DR Δ SS cross (Table 1). Thus, despite having substrates that with matched replication efficiencies, the minisatellite DNA failed to function as a hotspot in COS-1 cells.

There were three possible explanations for the lack of minisatellite-stimulated recombination in COS-1 cells. First, some aspect of replication of the substrate molecules might interfere with the hotspot activity that we observe between non-replicating substrates. Second, simian cells might fail to produce a recombinogenic factor that interacts with the minisatellite DNA. And third, the simian cells might produce a factor that interferes with the interaction of such a recombinogenic factor.

Minisatellite DNA Stimulates Recombination Between Nonreplicating Substrates in Simian Cells

The cell line CV-1 is the parental cell line of COS-1 cells and, because it does not produce SV40 T antigen, it provides an isogenic simian cell line for the analysis of hotspot function. Since CV-1 cells do not support substrate replication, we used a slightly different recombination assay than that used for the COS-1 cells (Fig. 2). The DL and DR molecules were introduced into the cells by calcium phosphate precipitation. In a certain proportion of the cells recombination occurred to generate a selectable, wild-type *neo* gene. The plasmids then integrated randomly into the genome and, after a period in selective medium, gave rise to G418 resistant colonies. The recombinant frequency was then calculated relative to the frequency of colonies generated by transformation with intact pSV2neo, which adjusts for the efficiency of DNA uptake and incorporation into the genome.

When we cotransfected CV-1 cells with DL and DR we obtained a recombinant frequency of 1.4×10^{-3} , demonstrating that CV-1 cells can catalyze recombination between nonreplicating recombination substrates (Table 2). In the DLSAT $\times$ DRSAT cross, the recombinant frequency was 54×10^{-3} , which revealed a 39-fold hotspot activity resulting from presence of the minisatellite DNA (Table 2). Similar results were obtained with the human bladder carcinoma cell line EJ (Table 2) (49), as well as with a variety of other mammalian cell lines (unpublished observations). We conclude that hypervariable minisatellite DNA can function as a hotspot in simian cells, as well as in other mammalian cells. This demonstrates that simian cells do contain the necessary factors to activate the minisatellite DNA recombination hotspot, and that the failure to observe hotspot activity in COS-1 cells is due to the presence of T antigen or some other aspect of replication of the recombination substrates.

T Antigen Abolishes Recombination Hotspot Activity of Minisatellite DNA Within Nonreplicating Substrates

The failure of the minisatellite DNA to function as a hotspot in COS-1 cells was due either to the presence of the T antigen, or the replication of the substrates. To distinguish between these two possibilities, we determined the recombinant frequencies in the pSVCT3-transformed human cell line CCD156. These cells actively produce a mutant T antigen that is capable of binding to and unwinding the DNA at the T antigen binding site, but is incapable of supporting the replication of plasmids containing the SV40 origin of replication (72).

CCD-156 cells were capable of catalyzing recombination between the DL and DR substrates and produced a recombinant frequency (2.1×10^{-3}) similar to those obtained with the human EJ cells and the simian CV-1 cells (Table 2). However, unlike EJ and CV-1 cells, the CCD-156 cells were unable to activate the minisatellite hotspot in the DLSAT $\times$ DRSAT cross (Table 2). We conclude that binding of SV40 T antigen immediately adjacent to the minisatellite DNA abolishes its recombination hotspot activity.

DISCUSSION

Homology Search Is Not Rate-Limiting for Episomal Substrates in Mammalian Cells

In order for chromosomes to undergo recombination, the homologous DNA sequences must find one-another and become aligned. In some organisms, discrete DNA sites on chromosomes are inferred to have an important role in the pairing of homologs during meiosis (73–75). In other organisms, such as budding yeast, a genome-wide (i.e., chromosome nonspecific) homology search probably occurs to bring chromosomes into register. Multiple, unstable interactions likely test for sequence homology between chromosomes, leading to progressively more stable interactions and, ultimately, full chromosome pairing (76, 77). In this “kissing” model, the machinery of recombination tests homology between segments, and, when homology is detected, promotes progressively more intimate interactions. While full homolog pairing is not a general feature of mitotic recombination, interaction between homologous chromosomes and sister chromatids does occur to affect recombinational repair of DNA damage (78, 79).

The homology search mechanism in budding yeast is very efficient. Recombination between ectopic loci, on the same or different chromosomes, occurs at a relatively high frequency (80). Furthermore, the rates of recombination between two ectopic alleles or those between multiple ectopic alleles at clustered or dispersed locations are very similar (81). We found that altering the steady state substrate concentration of episomes replicating in mammalian cells by an order of magnitude did not significantly change the recombinant frequencies

(Fig. 3, Table 1). In addition, the recombinant frequencies from crosses of replicating substrates (Table 1) were at most 6-fold higher than the recombinant frequencies from isogenic crosses in which replication did not occur (Table 2), despite major differences in both substrate persistence and substrate concentrations. We infer that the homology search is also very efficient in mammalian cells and is not a rate-limiting step of recombination. While such analyses have not been reported for loci within mammalian chromosomes, our conclusion is supported by similar gene targeting efficiencies at unique and multicopy (800 copies) chromosomal targets in somatic cells (82).

Protein-DNA Interaction Is Required for Recombination Hotspot Activity of Minisatellite DNA

Eukaryotic homologous recombination is a complicated process that involves multiple components and biochemical activities. To seek evidence that binding of specific proteins to minisatellite DNA was responsible for hotspot function, it was necessary to develop an assay system that could be used within living cells. Our experimental system was configured to test whether a site-specific DNA helicase could compete with an adjacent minisatellite DNA sequence and abolish hotspot function (Fig. 1).

The SV40 T antigen binds avidly to the SV40 replication origin (83, 84) and, in the presence of ATP, MgCl₂, and single-strand DNA binding protein, it unwinds DNA at its binding site (85, 86). Furthermore, the T antigen contains an intrinsic DNA helicase that unwinds DNA flanking the T antigen binding site (85, 87–89). Thus, binding to the DNA, opening of the DNA at that site, and helicase activity that unwinds the DNA flanking the binding site are provided solely by the T antigen. Subsequently, other host factors are required to complete the replication process (90). The preinitiation complex, composed of partially unwound DNA stabilized by single-strand binding protein, should compete effectively with any proteins binding to adjacent DNA sites. Since the minisatellite DNA was placed 8 bp away from the T antigen binding site (Fig. 1), such competition would be revealed as the elimination of hotspot function.

Our results and a model for function are summarized in Fig. 4. A certain level of basal recombination occurs between the substrates. This basal recombination rate is not affected by binding of T antigen. Insertion of hypervariable minisatellite DNA creates a recombination hotspot, provided that T antigen is absent, in all mammalian cell types tested. However, binding of SV40 T antigen immediately adjacent to the minisatellite DNA abolishes hotspot function, regardless of whether or not the substrates are replicating. The adjacently-bound T antigen, therefore, in some way prevents the recombinogenic activity of the minisatellite DNA. The simplest interpretation is that the T antigen is competing for site occupancy with minisatellite DNA binding proteins whose binding to the minisatellite DNA activates the recombination hotspot.

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Fig. 1.

Homologous recombination substrates Circular plasmids are presented as linear schematics for the sake of clarity. (a) The recombination substrates were derived from pSV2neo which contains the *neo* gene (white box) that can be expressed in *E. coli* and in mammalian cells due to the presence of the SV40 virus early promoter and poly(A) addition sequences (black boxes). In addition, the plasmids can replicate as episomes in mammalian cells expressing SV40 virus large T antigen due to the presence of the SV40 origin of replication (56). Deletion left (DL) and deletion right (DR) were created by removing *NarI-NarI* and *NaeI-NaeI* restriction fragments, respectively (57). (b) Expanded detail of substrates. The hypervariable minisatellite (SAT) recombination hotspot was cloned in the position of the *StuI-HindIII* fragment in pSV2neo and the various deletion plasmids (49). This places the minisatellite DNA, and its binding proteins (MsBP) (53), 8 bp away from the T antigen binding site.

Fig. 2.

Strategy to determine recombinant frequencies. The assay relies upon homologous recombination to generate a dominant, selectable marker gene (*neo*⁺) from two nonfunctional, nonreverting deletion genes that are cotransfected into the same cell. Both reciprocal (X) and nonreciprocal (not shown) recombination events can occur (48, 49). For cells incapable of supporting plasmid replication, the selectable gene integrates randomly into the genome and confers cellular resistance to G418. The number of G418 resistant colonies generated is directly proportional to the recombinant frequency. For cells permitting plasmid replication the episomal DNA is purified and transfected into recombination deficient *E. coli*, which reveals the recombinant frequency (*neo*⁺, kanamycin resistant) relative to the transformation efficiency (ampicillin resistant).

Fig. 3. Replication efficiencies of substrates. (a) Autoradiograph of replicated DNA isolated from COS-1 cells. Cells were cotransfected with the internal replication control plasmid pX21ori and the various recombination substrates. After 56 h of incubation low molecular weight DNA was extracted, linearized by *SalI* restriction enzyme digestion, and the unreplicated input DNA was destroyed by a five-fold excess (relative to *SalI*) of *DpnI* restriction enzyme. Southern blotting revealed the relative amounts of replicated pX21ori and the pSV2neo derivatives. The replication efficiency of each plasmid was determined by laser densitometry of the autoradiograph. (b) DNA sequence changes at the T antigen binding site and relative replication efficiencies of the recombination substrates. The substrates pDLΔSH and pDLΔSS (59) have replication efficiencies comparable to those of pDLSAT and pDRSAT, permitting a direct comparison of recombinant frequencies between pairs of substrates with matched replication rates.

Fig. 4.

Model of function. A normal, basal level of recombination occurs in cells lacking the minisatellite DNA hotspot and this basal frequency of recombination is essentially unaffected by T antigen binding or substrate replication. In the absence of T antigen, interaction of minisatellite DNA binding proteins (Msbp) (53) promotes recombination in nearby DNA intervals. Binding of T antigen displaces the minisatellite DNA binding proteins and renders the hotspot nonfunctional.

Table 1

Recombinant Frequencies Between Deletion Plasmids Replicating in COS Cells

Input DNA ^a	No. expts.	Kan ^r /Amp ^r colonies ^b	Recombinant frequency($\times 10^3$)	Hotspot ratio ^c
DL + DR (separate transfections) ^d	1	0/23,200	≤ 0.04	
DLSAT + DRSAT (separate transfections) ^d	1	0/24,200	≤ 0.04	
DL + DR	3	568/63,400	9.0	1.0
DLSAT + DRSAT	3	248/38,500	6.4	0.7
DL Δ SH + DR Δ SS	3	402/54,500	7.4	1.0 (0.8) ^e
DLSAT + DR Δ SS	3	383/38,600	9.9	1.3
DL Δ SH + DRSAT	3	230/51,400	4.5	0.6
DLSAT + DRSAT	3	253/37,000	6.8	0.9

^a A 10 μ g portion of each substrate DNA was introduced into 2×10^6 COS cells per 100 mm plate for each experiment.

^b Low molecular weight DNA obtained from the COS cells was transformed into *E. coli*. Part (0.1 volume) of each culture was used to determine the transformation efficiency (Amp^r colonies) and the remainder (0.9 volume) was used to determine the recombinant frequency (Kan^r colonies).

^c Ratio of the recombinant frequency relative to the recombinant frequency between plasmids lacking the hypervariable minisatellite DNA insert.

^d Recombination substrates were individually transfected in parallel into COS cells. Low molecular weight DNA was isolated from the two transfections and mixed prior to transformation of *E. coli*.

^e Ratio of the recombinant frequency relative to that between unmodified substrates containing an intact SV40 T antigen binding site.

Table 2

Recombinant Frequencies Between Nonreplicating Deletion Plasmids

Cell line ^a	Input DNA ^b	No. expts.	Total colonies/total μ g of DNA	Colonies/ μ g of DNA	Recombinant frequency ($\times 10^3$) ^c	Hotspot ratio ^d
EJ	pSV2neo	4	753/12	62.8		
	pSV2neoSAT	4	784/12	65.3		
	DL	4	0/80	≤ 0.02		
	DR	4	0/80	≤ 0.02		
	DLSAT	4	0/80	≤ 0.02		
	DRSAT	4	0/80	≤ 0.02		
CV-1	DL + DR	4	45/200	0.225	3.6	1.0
	DLSAT + DRSAT	4	579/190	3.05	49	14
	pSV2neo	4	515/8	64.4		
	pSV2neoSAT	4	503/8	62.9		
	DL + DR	4	18/200	0.090	1.4	1.0
	DLSAT + DRSAT	4	701/200	3.51	54	39
CCD156	pSV2neo	4	989/8	112		
	pSV2neoSAT	2	307/4	76.8		
	DL + DR	4	46/200	0.230	2.1	1.0
	DLSAT + DRSAT	4	62/200	0.310	2.8	1.3

^aEJ and CV-1 cells do not produce SV40 T antigen, CCD156 cells have been transformed with pSVCT3 and constitutively produce SV40 T antigen.^bIn each experiment 1 μ g of wild-type pSV2neo DNA or 10 μ g of each substrate DNA was transfected into 1×10^6 cells on a 60 mm dish.^cCalculated as (G418^r colonies/ μ g DL + DR)/(G418^r colonies/ μ g pSV2neo).^dRatio of the recombinant frequency relative to that obtained from the DL + DR cross.