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Genetics

Recombination of transfected DNAs in vertebrate cells in culture

(retrovirus/spleen necrosis virus/cloned DNA)

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ABSTRACT **We studied the frequency of homologous and illegitimate recombinations between transfected noninfectious retroviral DNA molecules in chicken embryo fibroblasts. The frequency of recombination was determined by the formation of infectious virus and varied with the extent of homology between the DNA molecules at the site of recombination, but only when there were regions of homology surrounding this region. While homologous recombination led to the formation of wild-type virus, illegitimate recombination resulted in formation of infectious virus with alterations at the site of recombination. Apparent homologous recombination was also observed between transfected and chromosomal DNAs in D17 dog cells.**

Homologous recombination and illegitimate recombination are normal cellular processes involved in genetic alterations and rearrangements. Both have been shown to occur in vertebrate cells in culture (see, for example, refs. 1–5).

We and others have been using transfection to study recombination in somatic cells (1, 6–8). It has been noted that the transfected DNA forms large concatemers, presumably as a result of ligation, recombination, or both (9). Recombination after transfection appears to involve both homologous and illegitimate recombinations. Homologous recombination between input DNAs is very frequent (8, 10, 11). Homologous recombination between transfected DNA and chromosomal DNA sequences has also been suggested to explain the expression of complete transplantation antigens by mouse L cells transfected with cloned DNA containing truncated genes for the class I genes of the major histocompatibility complex of mouse (12). Recombination between endogenous and exogenous simian virus 40 (SV40) genes has also been observed in SV40-transformed monkey CV-1 cells (13).

Illegitimate recombination during transfection also occurs at a high frequency (14, 15).

We wished to quantify the efficiencies of homologous and illegitimate recombinations between pairs of exogenous DNAs and of recombination between exogenous DNA and stable, presumably chromosomal, DNA in a system in which we could study rare events and control for the efficiency of transfection. Our experiments involve noninfectious DNA clones of spleen necrosis virus (SNV), an avian retrovirus, that contain different deletions or substitutions in the viral DNA. Successful recombination was assayed by the recovery of infectious virus DNA after transfection with DNA of two noninfectious viral DNA clones.

MATERIALS AND METHODS

Cells. Chicken embryo fibroblast cells were grown in modified Eagle's medium with 20% tryptose phosphate broth, 2% calf serum, and 3% fetal bovine serum. The canine cell line, D17 (from American Type Culture Collection), was grown in modified Eagle's medium with 7% fetal bovine serum (16). Selection for neomycin resistance was carried out in medium containing the gentamycin-related antibiotic G-418 (GIBCO) at 400 μg/ml (17). All cells were maintained at 37°C in a humidified CO_2 incubator.

Transfection and Assay of Infectious Virus. Transfection of recombinant DNAs was carried out by the calcium phosphate precipitation method of Graham and van der Eb (18). Transfected mammalian cells were usually exposed to 2 μM chloroquine when the DNA was present and were treated with 15% glycerol for 45 sec when the DNA was removed (19, 20). Three to 5 days after transfection, virus was harvested and was used to infect fresh chicken embryo fibroblast cells. Production of infectious virus was monitored by the appearance of cytopathic effects and DNA polymerase activity (21). Controls for contamination of plasmids or cells were included in all experiments.

Construction of Recombinant DNAs. pPB101 was constructed by inserting the permuted form of SNV sequences, pSNV-60B (22), into the *Sal* I site of pSW210. [pSW210 is pBR322 containing one long terminal repeat (LTR) with 40 and 250 base pairs (bp) from SNV on the 5′ and 3′ sides, respectively.] pSW279, pSW227, pSW272, pSW249, clone 5′ to *Hin*dIII of SNV, and clone 3′ from *Sal* I of SNV were described previously (Fig. 1; refs. 6, 16, 22). pSW264 consists of SNV sequences from the 5′ LTR up to 676 bp and reticuloendotheliosis virus type A (REV-A) sequences from 860 bp to the end of virus DNA (coding sequences and 3′ LTR). pSW280 was derived by deleting the *Xba* I (1.02 kbp) to *Xba* I (1.95 kbp) fragment from pPB101. pPB160 is a recombinant DNA containing the herpes simplex virus type 1 thymidine kinase gene (HSV *tk* gene) (with deletions in the promoter and termination sequences for *tk* RNA synthesis), inserted between the *Bgl* II (2.0 kbp) and *Bgl* II (6.6 kbp) cleavage sites in pPB101. p3′ from *Xho* I is a subclone of pPB101. It was constructed by digesting pPB101 with *Xho* I and cloning in the *Sal* I site of pBR322.

pME111 is a recombinant DNA molecule containing the HSV *tk* gene and DNA sequences coding for neomycin resistance (from Tn*5*) inserted in a retrovirus vector constructed with SNV (M. Emerman, personal communication).

Cell Clones. Cells of the dog cell line D17 were cotransfected with a noninfectious virus DNA with two LTRs and containing a large deletion of viral sequences and a substitution of the HSV *tk* gene (pSW227) and a selectable marker, SV2-*neo*^{r} (23). Cell clones resistant to G-418, among them D17(SW227)B4 and D17(SW227)B5, were isolated.

D17(ME111)Hf cells were a gift of M. Emerman (University of Wisconsin). They were constructed as follows: D17-C3, a helper cell line for avian reticuloendotheliosis viruses (16), was transfected with 5 μg of pME111 DNA (Fig. 1). Trans-

Abbreviations: SNV, spleen necrosis virus; REV-A, reticuloendotheliosis virus type A; HSV, herpes simplex virus; LTR, long terminal repeat; bp, base pair(s).

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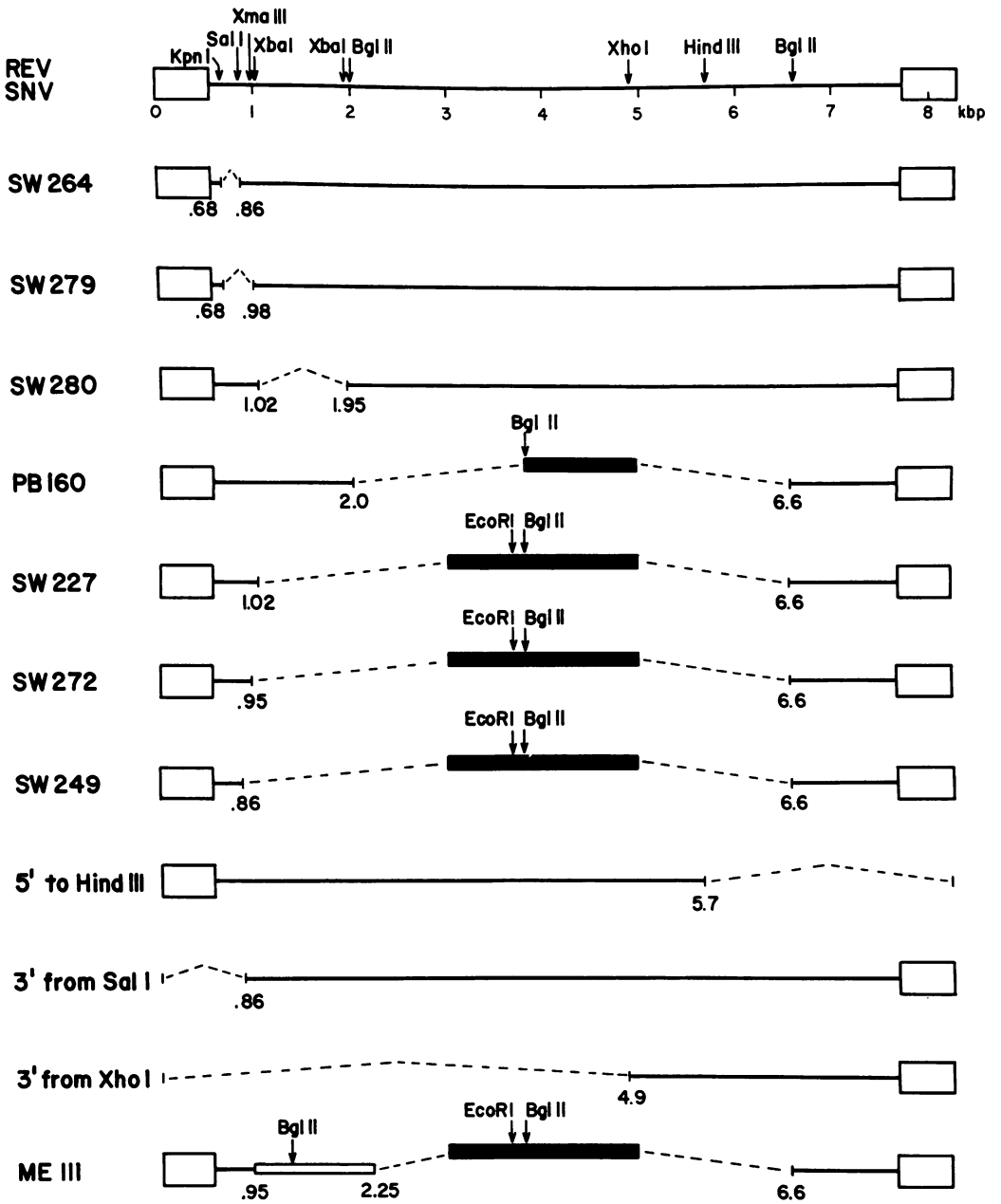

FIG. 1. Structures of viral DNAs: Maps of viral DNAs from the plasmids used in this paper. Infectious REV-A or SNV is shown at top. (These two viruses are very similar.) The rest of the DNAs are deleted or substituted as shown. The restriction enzyme cleavage sites used for the deletions or substitutions are shown in the top REV/SNV map and in the substitutions. The numbers under the maps of the noninfectious viruses indicate the boundaries of the deletions or substitutions in viral DNA. The deletions are indicated by broken lines rising above the maps; the substitutions, with filled-in bars indicating the herpes simplex virus (HSV) type I thymidine kinase gene (*tk*) without sequences for the end of its mRNA and in PB160 without its promoter and with a thin open bar indicating the *neo*^{r} gene; the LTRs, with open boxes; and the rest of the viral sequences, with solid lines. In some of the constructs 50 bp of SNV sequence next to the *tk* gene are not shown. The encapsidation sequence, E, maps between the *Kpn* I and *Sal* I cleavage sites (0.68 to 0.86 kbp) (22). The splice donor site maps 12 to 19 bp 5′ of the *Sal* I cleavage site (16).

fection of D17-C3 cells with DNA clones derived from SNV yields defective virus free of any wild-type helper virus (16). Medium was collected 20 days after transfection and was used to infect fresh D17 cells. Cell colonies resistant to G-418 at 400 μg/ml were picked. Several such D17(ME111) clones were used.

DNA Analysis. Procedures for extraction of DNA, Southern transfer, and nucleic acid hybridization were described previously (22).

RESULTS

Recombination Between Pairs of Exogenous DNAs. To study the efficiency of homologous recombination between exogenous DNAs, we transfected chicken cells with a constant high amount of one noninfectious viral DNA (10 μg/ml) as a carrier and dilutions of a second noninfectious viral DNA (6). The two DNAs were deleted forms of infectious SNV with two LTRs (see Fig. 2*A*). One DNA of each pair had its encapsidation sequences (*E*) and splice donor sequences deleted. The pairs of DNAs were selected to have different amounts of overlapping sequences in which homologous recombination would lead to formation of an infectious virus (region *b* in Fig. 2*A*), from 1.0 kbp for pSW279 and pPB160 to 40 bp for pSW279 and pSW280. Reciprocal crosses reversing which defective DNA was diluted and which acted as carrier gave similar results. In parallel we

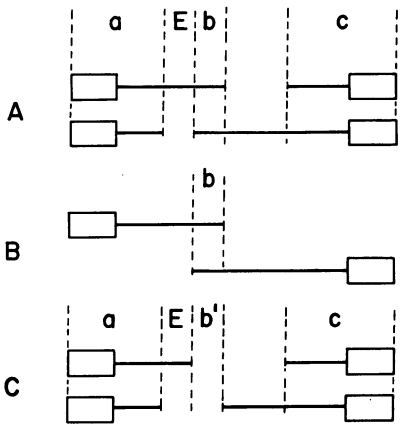

FIG. 2. Diagram of pairs of noninfectious viral DNAs: Schematic drawings (not to scale) of pairs of noninfectious viral DNAs used in recombination experiments described in text. Recombination in region *b* is homologous and leads to formation of an infectious virus. *E* is encapsidation sequences. Open boxes are LTRs; lines, rest of viral sequences. *A* represents pSW279/pPB160, pSW280/pSW264, and pSW279/pSV280. *B* represents p5′ to *Hin*dIII/p3′ from *Sal* I and p5′ to *Hin*dIII/p3′ from *Xho* I. *C* represents pSW279/pSW272 and pSW279/pSW249.

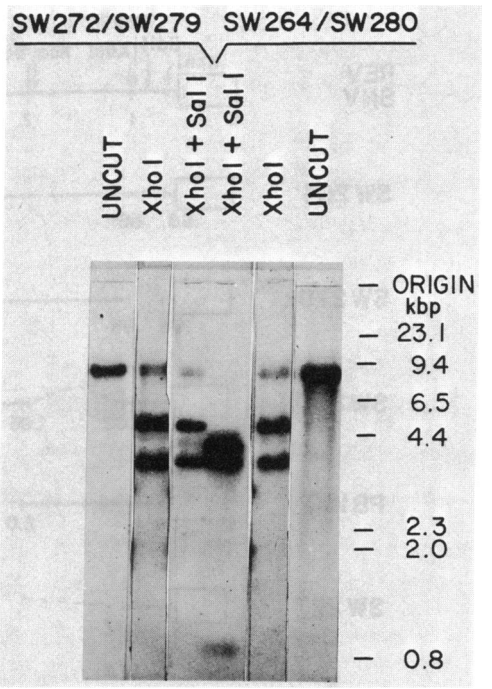

FIG. 3. Southern analysis of unintegrated viral DNA isolated from chicken embryo fibroblasts infected with virus harvested from cultures of chicken embryo fibroblasts transfected with a mixture of either pSW272 and pSW279 DNAs or pSW264 and pSW280 DNAs. The DNA was digested with *Xho* I, with a mixture of *Xho* I and *Sal* I, or not digested (uncut). The viral DNA was hybridized to ^{32}P-labeled SNV DNA. [REV-A DNA present in the reaction mixture of *Xho* I-digested SW272/SW279 DNA was cut by *Sal* I, indicating the absence of any inhibitory factors in this reaction mixture (data not shown).]

transfected cells with dilutions of DNA of an infectious clone of virus, using calf thymus DNA as a carrier. The results, presented in Table 1, indicate that the efficiency of recombination to yield infectious virus relative to recovery of virus from infectious clones varied with the size of the region in which recombination would lead to formation of an infectious virus from 50% at 1 kbp to 0.1% at 40 bp. The recovered virus is apparently wild type as judged by restriction enzyme analysis (Fig. 3, SW264/SW280).

To determine whether this relationship between efficiency of recombination and size depended only upon the length of the sequences in which recombination would lead to the formation of infectious virus, we transfected in a similar manner pairs of DNAs in which each DNA had only a single LTR (see Fig. 2*B*). The infectivity was determined in the same manner as described above and is presented in Table 1. [Similar results were reported earlier (6).] The relative efficiency of recombination of these DNAs per size of overlap was lower than seen above and surprisingly did not vary with the extent of the overlap. Thus, the efficiency of recombination appears to be linear with the extent of the overlap only when surrounding regions of homology are present in the two DNAs.

Table 1. Recombination after transfection

DNA clones	Regions of homology*, kbp			Infectivity, infectious units/μg
	a	*b*	*c*	
pSW253 (REV-A)		8.3		10^5
pSW279/pPB160	0.68	1.0	1.7	5×10^4
pSW280/pSW264	0.68	0.16	6.35	10^3
pSW279/pSW280	0.68	0.04	6.35	10^2
pSW279/pSW272	0.68	−0.03†	1.7	1–10
pSW279/pSW249	0.68	−0.12†	1.7	0.1
p5′ to *Hin*dIII‡/ p3′ from *Sal* I§	0	4.8	0	10^3
p5′ to *Hin*dIII‡/ p3′ from *Xho* I§	0	0.80	0	10^3

DNAs whose structures are shown in Fig. 1 were used in pairwise combinations for cotransfection of duplicate cultures of chicken fibroblasts. In each pairwise combination, one DNA had its encapsidation sequences deleted (*E* in Fig. 2). In each case, one DNA was at a constant concentration of 10 μg/ml and the concentration of the other was varied. The infectivity is infectious units/μg of DNA of the diluted clone and represents the average of several experiments.

*a, b, and c correspond to regions marked in Fig. 2.

†Extent of deletion common to both molecules of cotransfecting DNA at the site of recombination, *b′* in Fig. 2*C*.

‡Plasmids were digested before transfection with *Hin*dIII to linearize the DNA. (Each clone contains only part of the viral DNA and a single LTR.)

§Plasmids were digested before transfection with *Sal* I to linearize the DNA. (Each clone contains only part of the viral DNA and a single LTR.)

To determine the relative efficiency of illegitimate recombination, we carried out similar experiments with pairs of noninfectious virus DNAs, each with two LTRs, in which there were overlapping deletions in a nonessential region of the two viruses (see Fig. 2*C*). Recombination to give a viable product has to occur in the region of DNA between the encapsidation site, E, present in SW249 or SW272 and the deletion (about 10 bp in SW249 and 100 bp in SW272) and in sequences between the *Xma* III site, 0.980 kbp, and the start of the *gag* gene at 0.985 kbp in SW279 (5 bp) (16, 22, 24). Recombination was observed in these crosses, but at a much lower relative efficiency than seen for homologous recombination (Table 1). When the overlapping deletion was 30 bp (pSW279 and pSW272), the relative efficiency was 10^{-4} to 10^{-5}, and when the overlapping deletion was 120 bp (SW279 and SW249), it was 10^{-6}. Moreover, the virus recovered had a larger deletion than the parental virus containing E. The *Sal* I site at 0.86 kbp was absent from the virus recovered after transfection with pSW279 and pSW272, while the *Kpn* I site at 0.68 kbp was present (Fig. 3 and unpublished data).

Recombination Between Exogenous DNA and Stable, Presumably Chromosomal, DNA. Dog cell clones containing vi-

Table 2. Infectivity after recombination with stable cellular DNA

Cell clone	Infectivity, infectious units/μg			
	pSW264	pSW279	3′ from *Sal* I	REV-A
D17(SW227)B4	1	1	1	300
D17(SW227)B5	10	1	ND	2000
D17(ME111)Hf2	10	0.2	ND	500
D17(ME111)Hf3*	1	ND	ND	100

Cell clones were transfected with plasmid DNAs at various concentrations. The formation of infectious virus was determined. The values are the average of several experiments. ND, not done.
*One of five other D17(ME111) clones was also positive after transfection with 5 μg of pSW264.

ral DNA that contained deletions [D17(SW227) cell clones] were transfected with various concentrations of DNA of different noninfectious virus DNA clones with deletions that did not overlap with the deletion in the resident noninfectious virus DNA. pSW264 and pSW279 have a 160- and a 40-bp region of homology, respectively, with the resident virus DNA (SW227) in which recombination will give rise to infectious virus (see Fig. 2*A*, region *b*). (In both cases, the encapsidation sequences were deleted from the transfecting DNA so that any recombination had to involve the incoming DNA or RNA transcribed from it, not virus.) The efficiency of recombination was determined by comparing the lowest concentration of noninfectious virus DNA that gave rise to infectious virus to the lowest concentration of DNA from infectious virus DNA transfecting the same cells in parallel. Two cell clones, B4 and B5, yielded infectious virus after transfection with deleted noninfectious virus DNA (Table 2). The relative efficiency was about 0.1 to 1% that of recovery of infectious viral DNA after transfection with an infectious clone. In addition, recombination was also observed when the transfected DNA did not have regions of homology on both sides of the region in which recombination would give rise to infectious virus (plasmid 3′ from *Sal* I; see Figs. 1 and 2*B*).

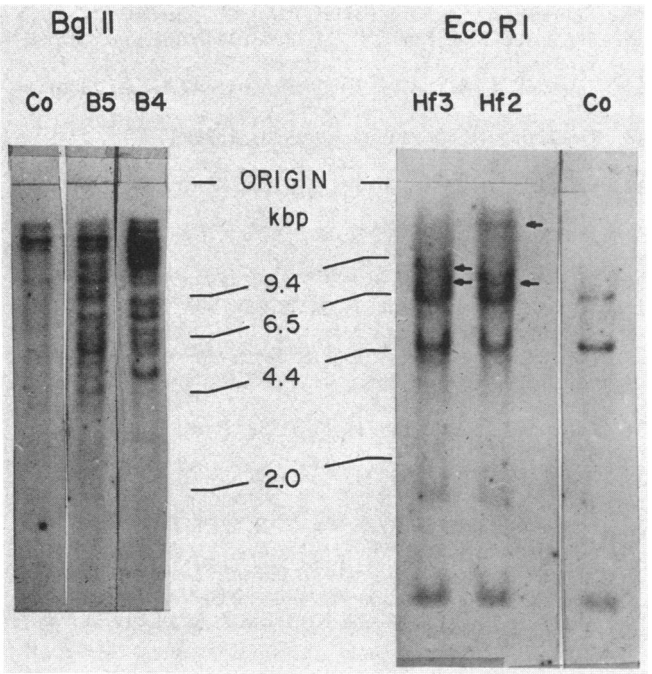

FIG. 4. Southern analysis of chromosomal DNA isolated from D17 cells and D17 cell clones. High molecular weight DNAs from D17 and D17(SW227)B5 and B4 were digested with *Bgl* II, and DNAs from D17 and D17(ME111)Hf 3 and 2 were digested with *Eco*RI, which cuts once in the middle of the provirus. Arrows next to Hf lanes indicate integrated copies of ME111 DNA. Lanes with D17 DNA are labeled Co. The viral DNA was hybridized to ^{32}P-labeled SNV DNA.

To determine the state of the resident viral DNA in the cells giving rise to infectious virus after transfection, high molecular weight DNA was prepared and analyzed. There were several copies of the SW227 DNA in a high molecular weight form in D17(SW227) clones B4 and B5 (Fig. 4). D17 cells, in addition, also have DNA sequences which are homologous to non-*env* sequences present in SNV (M. Emerman, personal communication).

Clones of D17(ME111) cells were also transfected with the noninfectious deleted DNA, pSW264. These cells differ from B4 and B5 in that they were isolated after infection rather than transfection (see *Materials and Methods*). Therefore, the noninfectious viral DNA in these cells is integrated into the chromosomal DNA as a provirus. The cells contain one or two proviruses of the defective virus ME111 (Fig. 4). Infectious virus was recovered from three of seven clones at an efficiency of about 1% relative to transfection with an infectious DNA clone of REV-A (Table 2). The virus recovered from clones Hf2 and 3 transfected with pSW264 was used to infect chicken embryo fibroblasts. The recovered virus was the size of wild-type virus and gave wild-type *Sal* I fragments after digestion (data not shown).

DISCUSSION

We performed studies to quantify the relative efficiencies of homologous and illegitimate recombinations in eukaryotic cells after transfection with DNA by using the calcium phosphate precipitation technique. In this procedure, cells take up large amounts of DNA in complexes termed "pecalosomes" or "transgenomes" (9).

Noninfectious DNA molecules carrying deletions at different sites in the genome of a cloned retrovirus were studied. In all cases the deletion in one of the viral DNAs covered the encapsidation sequence. Therefore, viral RNA from this clone could not be packaged (22). The concentration of the DNA of one of the deleted viruses was varied while the other was maintained constant at a saturating concentration. By assaying in parallel the recovery of wild-type virus from an infectious DNA clone, we could determine the relative efficiency of recombination. Since the experiments were performed in cells that are permissive for virus propagation, virus obtained from low levels of recombination was amplified, thereby increasing the sensitivity of detection of rare recombinants.

Homologous Recombination. The relative frequency of homologous recombination observed in these studies varied with the size of the regions of homology, both at the site of recombination and at areas adjacent to it. The regions of homology can be divided into three parts, *a*, *b*, and *c* (Fig. 2). *b* is the site of potential recombination (crossover), and *a* and *c* are flanking homologous sequences.

The size of region *b* is most important in determining the relative efficiency of recombination when there are comparable flanking homologous sequences *a* and *c* (Table 1).

Pomerantz *et al.* (1) presented analogous results on recombination between pairs of nontransforming mutants of polyoma virus carrying different lesions.

When there were not flanking homologous sequences (Fig. 2*B*), the relative frequency of recombination was reduced to 1% of the control value (Table 1) (6). The efficiency, moreover, was independent of the extent of the homology (*b* in Fig. 2*B*). Comparing the results in Table 1, we see that the *a* and *c* regions of homology (Fig. 2) increase the efficiency of recombination over cases when one of these is missing. Similar results were also obtained for recombination between nonoverlapping deletions in the coding sequences of the HSV *tk* gene inserted in a retrovirus vector

constructed from SNV (unpublished observations).

Illegitimate Recombination. Illegitimate recombination occurred at a very low relative frequency (Table 1). The low relative efficiency of illegitimate recombination observed here is probably a reflection of the stringent requirement to produce infectious virus. In the absence of DNA sequence homology at the recombination site, recombination sites are probably distributed over the nonhomologous DNA sequences. However, recombinants that disrupt essential *cis*-acting sequences or viral coding functions are not viable.

In the pair pSW272 and pSW279, there is no DNA sequence homology in the region 0.68–0.98 kbp. The recombination site is, therefore, not defined. A consequence of this is that the recombinant virus has a deletion larger than the parental virus with *E*, pSW272 (see Fig. 3), indicating that the recombination did not take place at the edge of the deletions.

Ruley and Fried (25) analyzed the integrated viral sequences present in the polyoma virus-transformed cell line 82-Rat. They observed that multiple recombinational events occurred within small regions (*ca.* 50 bp) in the viral DNA and that very short homologous regions (3–4 bp) participated in joining two nonhomologous sequences. Inspection of the DNA sequences in the region of the illegitimate recombination reported here (24) revealed only one 3-bp homologous region in pSW279/pSW272 and none in pSW279/pSW249 that may have participated in this illegitimate recombination.

Recombination with Chromosomal DNA. Recombination between chromosomal DNA and exogenous DNA has been extensively documented in yeast. The integration of hybrid plasmids, containing both bacterial and yeast DNA, into the yeast genome occurs by homologous recombination (26). Increase in the total effective concentration of homologous sequences increases the frequency of homologous recombination (27–29).

A relative high frequency (about 1% of selected clones) of formation of complete gene products from truncated class I genes of the major histocompatibility complex was observed by Goodenow *et al.* (12) after transfection of mouse cells. This process was ascribed to homologous recombination between host chromosomal and donor class I DNA sequences. Our studies also suggest that recombination to yield wild-type virus occurs at a high relative efficiency between exogenous and cellular DNAs. Viable virus was produced after recombination between the transfecting plasmid pSW264 and a provirus as early as 3 days after transfection (data not shown).

Recombination must take place between molecules in one of these pairs: (*i*) input DNA with provirus, (*ii*) input DNA with RNA from provirus, (*iii*) RNA from input DNA with provirus, and (*iv*) RNA from input DNA with RNA from provirus. Since both the encapsidation and splice donor sites are absent from pSW264, virus cannot be formed, and hypothesis *iv* is unlikely unless there is some endogenous virus production. Recombination of RNA with DNA, hypotheses *ii* and *iii*, though theoretically possible, is unknown. Recombination at the level of DNA molecules, hypothesis *i*, is, therefore, most probable, perhaps driven by the high concentration of transfected DNA or a special location of transfected and provirus DNAs. [It also may be relevant that recombination of input DNA (pSW264 and pSW280) is apparently an order of magnitude more efficient in D17 cells than in chicken cells (data not shown).] Recovery of integrated recombinant progeny will be needed to test this hypothesis.

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1. Pomerantz, B. J., Naujokas, M. & Hassel, J. A. (1983) *Mol. Cell. Biol.* **3**, 1680–1685.
2. Stringer, J. R. (1981) *J. Virol.* **38**, 671–679.
3. Botchan, M., Stringer, J., Mitchison, T. & Sambrook, J. (1980) *Cell* **20**, 143–152.
4. Johnson, A. D., Barkan, A. & Mertz, J. E. (1982) *Virology* **123**, 464–469.
5. Woodworth-Gutai, M. (1981) *Virology* **109**, 344–352.
6. Miller, C. K. & Temin, H. M. (1983) *Science* **220**, 606–609.
7. Shapiba, G., Stachelek, J. L., Letsou, A., Soodak, L. K. & Liskay, R. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4827–4831.
8. Upcroft, P., Carter, B. & Kidson, C. (1980) *Nucleic Acids Res.* **8**, 2725–2736.
9. Perucho, M., Hanahan, D. & Wigler, M. (1980) *Cell* **22**, 309–317.
10. Birkner, K. L. & Sharp, P. A. (1983) *Nucleic Acids Res.* **11**, 6003–6020.
11. de Saint Vincent, B. R. & Wahl, G. M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2002–2006.
12. Goodenow, R. S., Stroynowski, J., McMillan, M., Nicolson, M., Eakle, K., Sher, B. T., Davidson, N. & Hood, L. (1983) *Nature (London)* **301**, 388–394.
13. Vogel, T., Gluzman, Y. & Winocour, E. (1977) *J. Virol.* **24**, 541–550.
14. Winocour, E. & Keshet, I. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4861–4865.
15. Subramani, S. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 1040–1052.
16. Watanabe, S. & Temin, H. M. (1983) *Mol. Cell. Biol.* **3**, 2241–2249.
17. Jimenez, A. & Davies, J. (1980) *Nature (London)* **287**, 869–871.
18. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–467.
19. Luthman, H. & Magnusson, G. (1983) *Nucleic Acids Res.* **11**, 1295–1308.
20. Stow, N. D. & Wilkie, N. M. (1976) *J. Gen. Virol.* **33**, 447–458.
21. Temin, H. M. & Kassner, V. K. (1974) *J. Virol.* **13**, 291–297.
22. Watanabe, S. & Temin, H. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5986–5990.
23. Southern, P. & Berg, P. (1982) in *Eukaryotic Viral Vectors*, ed. Gluzman, Y. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 41–45.
24. O'Rear, J. J. & Temin, H. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1230–1234.
25. Ruley, H. E. & Fried, M. (1983) *Nature (London)* **304**, 181–184.
26. Hinen, A., Hicks, J. B. & Fink, G. R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929–1933.
27. Szostak, J. W. & Wu, P. (1979) *Plasmid* **2**, 536–554.
28. Beggs, J. D. (1978) *Nature (London)* **275**, 104–109.
29. Stinchcomb, D. T., Struhl, K. & Davis, R. W. (1979) *Nature (London)* **282**, 39–43.