The Same Mammalian Replicon Yields Distinct Recombination Products in Different Cell Lines*

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We have observed previously that some chimeric replicons inclusive of a partly duplicated polyomavirus (Py) genome yield unit-length Py DNA (P155) at high frequency when transfected into normal or Py-transformed mouse cells. We demonstrate here that one such replicon generates either P155 or illegitimate recombination products in other mouse cells, transformed by simian virus 40. Use of the polymerase chain reaction indicates that each of the illegitimate products carried a different deletion, but that all deletions mapped within a rather well defined portion of the precursor replicon. Thus, these products were organized as if two hotspots for recombination existed in the Py late-coding region, one being located within or near one of the duplicated sequences characteristic of the chimeric replicon. Since this particular hotspot has already been shown to be involved in the generation of P155, the data reported here could indicate that a single recombination mechanism can yield either homologous (P155) or illegitimate products. How the DNA interacts with certain proteins, such as papovavirus large tumor antigen, could explain why one or the other type of product is formed.

It has been pointed out that illegitimate recombination events are more common in mammalian cells than in bacteria or yeast. In mammalian cells, homologous and nonhomologous recombination products are generated intramolecularly at comparable frequencies (1–4), whereas intermolecular recombination yields nonhomologous products more frequently than homologous ones (5). It has never been clear, however, whether the two types of products arise from two different mechanisms or a single one, since in the latter hypothesis variation of a single regulatory factor would be all that is needed to account for the observed results. As a tool for the study of recombination in cultured mouse cells, we use Rml (6, 7), a Py1-mouse hybrid replicon inclusive of 1.6 kb of mouse DNA (Ins) inserted between two 182-bp-long direct viral repeats (S repeats) in an otherwise unrearranged Py genome (Fig. 1A). When Rml is transfected into normal or Py-transformed mouse cells, recombination between the two S repeats yields unit-length Py DNA (P155) which, being readily amplified, is directly detectable on blots (8). However, when Rml is transfected into SV40-transformed NIH-3T3 cells, P155 is produced concurrently with other, larger or smaller, molecular species inclusive of Py DNA. We have attributed this altered pattern of recombination to the simultaneous binding, to Rml, of the Py LT encoded by the chimera, and the SV40 LT encoded by the resident viral genome (9). In agreement with this interpretation, the relative amount of the molecular species distinct from P155 is increased when the template for recombination is SVRml (Fig. 1B), a derivative of Rml in which a fragment of SV40 DNA encompassing the recombination origin (Ori) has been introduced with, as another consequence, a strong reduction of the whole molecule's ability for autonomous recombination (9). The fact that SVRml replicates and recombines differently from Rml suggests that both operations are dependent upon trans- and cis-acting functions common to both replicons. This conclusion is supported by two sets of observations. First, in Py-transformed mouse cells supplying an active Py LT, SVRml replicates and, more importantly, recombines as does Rml in normal mouse cells (9). Thus, substituting a constitutively expressed Py LT for a similarly expressed SV40 LT not only relieves the inhibition imposed by the SV40 Ori on the replication of SVRml, but also allows this molecule to recombine effectively into a single molecular species, P155. Secondly, derivatives of Rml were constructed in which the internal homology was raised from 182 bp to 1–2 kb: such replicons yield P155 even more readily than Rml, yet via crossovers occurring almost invariably within or near the S repeats characteristic of Rml (10). Therefore, the generation of P155 from Rml involves two recombination hotspots, one in each S repeat. Recombination is, however, dependent upon the position of the S repeats with respect to the Ori, as if hotspot activity was stimulated by a process traveling along the DNA, such as replication or transcription (10).

In the present report, we characterize the amplifiable recombination products arising in SV40-transformed NIH-3T3 cells from pSVI-1, a recombinant plasmid constructed by introducing the SV40 Ori into pl-1, that is Rml cloned in pBR322 (Fig. 2). As already noted for Rml and SVRml (9), pSVI-1 yields a whole collection of products in such SV40-transformed cells. Most of these products, designated here HRP (for heterogeneous recombination products), seem to result from intramolecular recombination between two sites, often both included in the Py DNA moiety of pSVI-1. Whereas one of these two sites lies near or within the late S repeat, or closer to the Py Ori, the other is frequently located near or within the early S repeat (Fig. 1). The fact that S repeats appear to act as recombination hotspots regardless of the nature of the product may indicate that P155 and HRP are generated by related mechanisms.
were incubated at 37 °C, first for 1 h in 5% CO₂, and then for 3 h in 60-70% confluent monolayer (in 100-mm Petri dishes), plates containing the cell lines used in this study has already been described (8, 9).

Cells, DNA Transfection, and Extraction—The origin of the different cell lines used in this study has already been described (8, 9). Transfections were carried out by the method of Sussman and Milstein (11) with minor modifications: after addition of 2 µg of DNA per 60-70% confluent monolayer (in 100-mm Petri dishes), plates were incubated at 37 °C, first for 1 h in 5% CO₂ and then for 3 h in 10% CO₂. Following dimethyl sulfoxide shock (10%, 2 min), incubation was continued at 33 °C for 3 or 6 days in Dulbecco's modified Eagle's medium supplemented with 5% calf serum. Low molecular weight DNA was extracted by the method of Hirt (12) and digested with DpnI prior to PCR, but generally not prior to Southern blotting (10, 16).

Plasmids—Recombinant pl-1 has already been described (8). The construction of pSVI-1 was as follows: SVRmI was released from pSVB1-20 by SalI digestion (9), then self-ligated, cut with BglII (Fig. 1B), and finally inserted at the BamHI site of pBR322 (Fig. 2B). Restriction enzymes (Pharmacia LKB Biotechnology Inc.) and T4 DNA ligase (Amersham Corp.) were used as recommended by the suppliers.

DNA Transfer and Hybridization—After gel electrophoresis, DNA was transferred onto nylon membranes (Hybond-N, Amersham Corp.), using the VacuGene blotting unit from Pharmacia (1961-S1). Hybridization was carried out as already described (7). Radioactive probes were prepared with the MPvirus Random DNA Labelling System from Amersham, using [α-32P]dCTP (≈3000 Ci/mmol, Amersham) as radioactive precursor.

PCR—In all cases, the DNA template was that provided by a Hirt supernatant from about 10⁶ cells. Prior to in vitro amplification, the extract was treated with DpnI to eliminate molecules having not replicated since transfection, and then with BglII to counterselect the template for recombination (besides the three BglII sites in pBR322, there is one near the SV40 Ori, Fig. 1B). Digestion by BglII, which should cleave putative recombination products near the Py Ori, was also performed on the P155 used as a positive control. Primers 548, 549, 597, and 598 (Fig. 3) were first obtained from K. Deugau (Queen's University, Kingston, Ontario) and then synthesized in our own facilities.

Fig. 1. Physical maps of RmI and SVRmI. A. RmI includes 1.63 copies of the DNA (53.5 kb) of the tsP155 mutant of Py (m), plus 1.6 kb of mouse DNA (L). The mouse insert (Ins) is included in the viral late-coding region (L) and bracketed by two direct, 182-bp-long viral S repeats (arrowheads). Cellular bp are labeled -1 to -1628 (counterclockwise), with bp -1 linked to viral bp 3092, and bp -1628 linked to viral bp 3273 (each S repeat includes bp 3092-3273). The viral early-coding region (E) extends clockwise from the Ori to approximately the unique SalI site. The S repeat immediately beyond that SalI site is called the early S repeat, the other the late S repeat.

B, SVRmI was obtained by inserting the Sau3A fragment of SV40 DNA inclusive of the Ori (Q) into the unique BglII site of RmI. Notice that SVRmI carries a BglII site. The numbering of viral bp used throughout the paper is that of Tooze (23).

Fig. 2. Physical maps of recombinant plasmids pl-1 and pSVI-1. Most of the symbols are those used in Fig. 1, except for the dotted areas indicating pBR322 DNA. pl-1 and pSVI-1 were obtained by ligating BamHI-digested pBR322 with, respectively, BglII-digested RmI, and BglII-digested SVRmI (see Fig. 1, and “Materials and Methods”).

MATERIALS AND METHODS

Cells, DNA Transfection, and Extraction—The origin of the different cell lines used in this study has already been described (8, 9). Transfections were carried out by the method of Sussman and Milman (11) with minor modifications: after addition of 2 µg of DNA per 60-70% confluent monolayer (in 100-mm Petri dishes), plates were incubated at 37 °C, first for 1 h in 5% CO₂ and then for 3 h in 10% CO₂. Following dimethyl sulfoxide shock (10%, 2 min), incubation was continued at 33 °C for 3 or 6 days in Dulbecco's modified Eagle's medium supplemented with 5% calf serum. Low molecular weight DNA was extracted by the method of Hirt (12) and digested with DpnI prior to PCR, but generally not prior to Southern blotting (10, 16).

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Fig. 3. PCR detection of recombination in pSVI-1. A, the portion of P155 inclusive of the oligonucleotides used as primers during PCR, as well as some restriction sites, are shown. Also shown are the cloned fragments of P155 which were converted into radioactive probes to be used in the characterization of recombination products (see Fig. 5). B, amplification of HRP: digestion with BglII cuts four times into the part of pSVI-1 toward which the 3' ends of the primers converge (see "Materials and Methods"). A single deletion removing precisely (Δ) the portion of pSVI-1 inclusive of those 4 BglII sites would leave uninterrupted the stretch of DNA shown here, with either 3.7 or 4.8 kb between the sites at which PCR could be initiated, depending on whether the 548–549 or the 597–598 pair was used. The amplified fragment would then be of four different origins (Py, mouse, SV40, and pBR322), shown here as in Fig. 2.
Distinct Recombination Products

pSVI-1 Yields HRP in SV40-transformed Mouse Cell Lines—In this study, we chose to deliver Rml and SVRml to the cell in the form of pl-1 and pSVI-1 (Fig. 2) for two practical reasons. First, pl-1 was already known to yield P155 after transfection even more effectively than Rml itself (8) and second, Py sequences in either pl-1 or pSVI-1 are unimpeded, so that excision and circularization of the insert prior to transfection becomes unnecessary (8). In preliminary experiments, both constructs were tested for replication and recombination in the four cell lines used previously: normal mouse 3T6 cells, SCOP-T1 cells (Py-transformed mouse cells synthesizing a wild-type Py LT), and R1-4 and Ori-1 cells, two mouse cell lines synthesizing different forms of SV40 LT (8). Examination of the species of DNA detectable in these cells 6 days after transfection lead to four main observations (Fig. 4). First, P155 is readily released in normal or Py-transformed mouse cells whether or not the template for recombination includes the SV40 Ori. Second, the extent of conversion of precursor into product (P155) is greater for pSVI-1 than for pl-1 as expected from our previous observations (see Materials and Methods). With P155 as template, one pair (548–549) would allow the synthesis of a 520-bp-long fragment, and the other (597–598) that of a 1428-bp-long fragment (Fig. 3A). No definite prediction regarding size could be made about fragments possibly amplified from HRP. However, as the DNA would be digested with BgII prior to PCR (“Materials and Methods”), one could easily anticipate which portion of pSVI-1 would be subjected to amplification, assuming that some deletions would remove subsets of the target sequences inclusive of all BgII sites (Fig. 3B).

Hirt supernatants of 3T6, Ori-1, and R1-4 cells transfected with pSVI-1 6 days previously were used to drive PCR reactions relying on the 597–598 pair of primers, and the resulting products were analyzed by the Southern procedure (Fig. 5A). In the case of 3T6 cells, a single fragment of 1428 bp annealing with a Py DNA probe was produced, confirming the validity of an approach already used successfully to detect the release

FIG. 5. Characterization of recombination products arising from pSVI-1. A, DNA amplified with primers 597–598. Purified viral DNA or DNA extracted from pSVI-1-transfected cells was subjected to PCR as described under "Materials and Methods." After electrophoresis of each of the PCR products the DNA was blotted and hybridized with a radioactive probe synthesized from cloned Py DNA. Individual PCR reactions had been directed by: pg (I) and 6) and B, the T7 sequencing kit from Pharmacia were used with

Note that the 0.46-kb band

merged or amplified HRP from SVRml involved at least one large deletion occurring opposite to the Py Ori (Fig. 1B). Possibly, the same type of deletion was also taking place in the case of pSVI-1. To explore this possibility, we decided to resort to PCR, using as primers two different pairs of oligonucleotides (see "Materials and Methods"). With P155 as template, one pair (548–549) would allow the synthesis of a 520-bp-long fragment, and the other (597–598) that of a 1428-bp-long fragment (Fig. 3A). No definite prediction regarding size could be made about fragments possibly amplified from HRP. However, as the DNA would be digested with BgII prior to PCR ("Materials and Methods"), one could easily anticipate which portion of pSVI-1 would be subjected to amplification, assuming that some deletions would remove subsets of the target sequences inclusive of all BgII sites (Fig. 3B).

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FIG. 4. Fate of pl-1 and pSVI-1 in various mouse cell lines. The DNAs were extracted by the Hirt method, digested with XhoI which introduces two closely spaced cleavages in either pl-1, pSVI-1, or P155 (Fig. 3), electrophoresed through a 1% agarose gel, blotted, and hybridized with a Py DNA probe. The first lane had been run with a radioactive ladder (L) and the last lane with 2×10–4 µg of XhoI-cleaved P155 (P, 5.3 kb). T, 3T6; S, SCOP-T1; R, R1-4; O, Ori-1. Note that as expected, pl-1 (11.5 kb) migrates faster than pSVI-1 (12.8 kb).
of P155 from larger precursor replicons (16). The same 1428-bp fragment was also noted for Ori-1 and R1-4 cells, which, however, differed strikingly from the 3T6 cells by the generation of several smaller species (compare lanes 3, 4, and 5 in Fig. 5A). As shown in Fig. 3A, the DNA amplified from P155 with primers 597–598 should be converted into two fragments upon digestion with Accl: a larger fragment encompassing the sequences that would be joined during recombination of pSVI-1 (963 bp, detectable by probe J) and a shorter fragment inclusive of the distal portion of the viral early region (465 bp, detectable by probe E). What happened when the DNA amplified from the Hirt extracts was cut by Accl, electrophoresed, and annealed with these probes is shown in Fig. 5B. Strikingly, whether the DNA subjected to Accl digestion had been found homogeneous (3T6) or heterogeneous (Ori-1 and R1-4), probe E detected only the 465-bp fragment expected from P155. In contrast, the J probe detected besides the 963-bp fragment characteristic of P155, a whole series of other materials: presumably one was amplified from P155 and the other from HRP, the two differing in the manner in which the viral early and late sequences were joined. As far as the uncleaved DNAs or their Accl fragments were concerned, it looked as if the HRP were generally smaller, rather than larger than P155 (Fig. 5), contrary to what the Southern blots of HRP junctions (see below). Nevertheless, the 548–549 pair was observed, even in the presence of smaller amplifiable fragments (16). Second, when primers 548–549 were used instead of primers 597–598 in a PCR such as that described above a fragment of only 520 bp was amplified from P155, and yet few or no significantly larger fragments were observed with Ori-1 and R1-4 cells (not shown; see also “Discussion”).

### Cloning and Sequencing of PCR Products

In an attempt to produce and clone junctional fragments characteristic of HRP the DNA amplified from Ori-1 and R1-4 cells was digested with suitable enzymes: Aval and PstI when PCR had been carried out with primers 548–549, XbaI and HindIII when primers 597–598 had been used (Fig. 3A). These enzymes had been chosen knowing that the amplified fragments from the HRP were generally shorter than that from P155, and that neither Ins nor the portions of SV40 or pBR322 DNA possibly incorporated in PCR products, contained any Aval, PstI, or XbaI sites (Fig. 3B). The fact that Ins contained a HindIII site (Fig. 3B) shall be addressed later. The restricted DNAs were ligated to similarly cleaved pUC13 DNA prior to transformation of E. coli DH5α, and the transformants were screened for plasmids (13) carrying Py DNA using a radioactive probe. From the 50 recombinant plasmids obtained, all those with inserts larger or smaller than that expected from the homologous recombinant product (P155) were characterized by the method of Sanger (“Materials and Methods”). Such work indicated that in 46 out of 50 instances recombination in pSVI-1 had involved a simple deletion.

### Deletions

24 inserts migrated on gel exactly as expected from the corresponding fragment of P155, suggesting an identity which for two inserts was verified by sequencing. Of these homologous deletions, 23 had been detected using primers 548–549, and only one with the 597–598 pair. This is not surprising, since further analysis indicated that using primers 548 and 549 for PCR was a way to select against amplification of HRP junctions (see below). Nevertheless, the 548–549 pair also allowed the detection of six nonhomologous junctions, three of which rendered the recombination product larger.

### Table I

Nonhomologous deletions entailed by pSVI-1 in Ori-1 and R1-4 cells

The deletions listed were those identifiable by PCR using either the 548–549 or the 597–598 pair of primers.

<table>
<thead>
<tr>
<th>Primers Inserta</th>
<th>End pointsb</th>
<th>Size of inserta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primers Inserta</td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>548–549</td>
<td>3038</td>
<td>3524</td>
</tr>
<tr>
<td>548–549</td>
<td>2906</td>
<td>3269</td>
</tr>
<tr>
<td>597–598</td>
<td>3102</td>
<td>368</td>
</tr>
<tr>
<td>597–598</td>
<td>3130</td>
<td>3272</td>
</tr>
<tr>
<td>597–598</td>
<td>1605</td>
<td>3232</td>
</tr>
<tr>
<td>597–598</td>
<td>1563</td>
<td>3248</td>
</tr>
<tr>
<td>Average insert size: 286</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table II

Characteristics of sequences surrounding the deletion end points

<table>
<thead>
<tr>
<th>Characteristica</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence homology at joining site</td>
<td>72.8</td>
<td></td>
</tr>
<tr>
<td>Sequence homology near joining site</td>
<td>67.5</td>
<td></td>
</tr>
<tr>
<td>Purine stretches</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Purine-Py stretches</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>A-T stretches</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>Topoisomerase I sites</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Multiple topoisomerase I sites</td>
<td>72.7</td>
<td></td>
</tr>
<tr>
<td>Topo I sites</td>
<td>6.3</td>
<td></td>
</tr>
</tbody>
</table>

a In each case the two sequences surrounding in pSVT-1 the deletion end points were aligned (see Fig. 5) and searched for homology and motifs which were those of Konopka (19).

b Proportion of sequences with the given characteristic.

Within 30-pb each side from the cross-over site, whether known or arbitrarily assigned (see Fig. 6). Only perfect alignments were considered.

Because of only partial overlap between this group and the above one, homology at or near the cross-over site was noted for 100% of deletions.

Within 5-pb each side from the cross-over site when known, or within 5 bp from the borders of the uncertainty region (see Fig. 6). Only stretches of 5 or more residues were counted.
Distinct Recombination Products

than P155 (Table I). But the striking common feature of these six junctions was that both of the coordinates mapped either within one of the S repeats or within less than 65 bp of it (see Table I). Yet, illegitimate recombinants with an intervening stretch of 1 or 2 kb between the two S repeats could conceivably have arisen from pSVI-1 (Fig. 3B) and, as already mentioned, would have been readily detected by our procedure. When junctions were amplified with primers 597-598, the involvement of the late S repeat in nonhomologous recombination became less obvious than that of other late viral sequences further toward the Py Ori (Table I). Even then, however, one coordinate was often found to lie within the early S repeat (Table I). No recombinant plasmid carried as an insert the DNA extending from the viral XbaI site at 2522 to the mouse HindIII site at -938 in Ins (Fig. 3B). Together with the data shown in Table I, this result should be taken as evidence that non-Py DNA remote from the S repeats is rarely if ever retained by HRP.

In Table II we show the distribution in relation to deletion end points of sequence motifs believed to play a role in nonhomologous recombination events. Our analysis indicated first and foremost that all of the stretches of DNA that had undergone recombination displayed limited homology at or near the presumptive crossover site. Even though the significance of this feature has been questioned (17, 18), the fact that it was observed for all deletions is unlikely to be fortuitous. Possibly, in the deletions which we have observed, homology was required for transitory alignment of sites between which recombination would occur. The homologies were of 2-4 bp when near the joining site and, when at the joining site, of 1-8 bp (with a mean of 3.5 bp). Our search for other features characteristic of nonhomologous recombination events was similar to that of Konopka (19), who made the distinction between true illegitimate recombination events and those involving transfected linear DNA. Whereas Konopka (19) found that the incidence of polypurine-pyro rimidine tracts and alternating purine/pyrimidine runs near the joining site was higher for illegitimate recombination events than for transfected linear DNA, we observed that this incidence was even higher for our HRP than for his illegitimate recombination events (Table I). In our case, the frequency of topoisomerase I cleavage sites near deletion end points was also quite high, as was that of Topo I* sites (Fig. 6), i.e. those sites in a position compatible with a direct involvement of the enzyme in the breaking-rejoining mechanism. All of these features of the sequences surrounding the deletion end points were thus quite typical of sites at which nonhomologous recombination is known to occur in mammalian cells.

FIG. 6. Features of sequences surrounding end points of deletions 4, 9, 12, and 10 (see Table I). The sequences are taken from that of CSP Py DNA (24) to which P155 is related or from that of Ins (6). In each instance, the sequence displayed above and that shown below are those mapping toward the early end and the late end of the deletion, respectively. Known crossover sites are indicated by a line connecting top and bottom sequences (\(\downarrow\)). When homology at the crossover site was a cause for ambiguity, the breakage-reunion coordinates were arbitrarily assigned to the nucleotides flanking the 5' border of the uncertainty region (\(\uparrow\); see also Table I). \(\uparrow\), nucleotides flanking the 3' border of the uncertainty region, the last ones possibly involved in the crossover. Homologies are boxed, A/T runs are underlined, and polypurine tracks are marked with black dots. Topoisomerase I sites are indicated in the 5' → 3' direction by arrowed lines. For deletion 10, a three-A/T bp gap filler was found at the joint.
Inversions—Four of the inserts cloned into pUC13 included ends that were those expected from the PCR protocol and subsequent cloning, but a central portion which consisted of not only deleted but also rearranged pSVI-1 DNA. In all cases this DNA consisted of the portion of Py DNA expected from the PCR protocol; in one instance Ins material was also present. Two inserts each carried an inversion with one end point mapping within (bp 3134) or near (bp 3082) one S repeat. For the two other inserts, extensive rearrangements rendered difficult any interpretation.

**DISCUSSION**

We believe that the results described above establish clearly that the same replicon, pSVI-1, yields different recombination products in two sets of cell lines of the same animal origin. First, sequencing of nonhomologous junctional fragments amplified by PCR suggests that they arise from authentic recombinant molecules and not from some artefact of PCR (20). Second, the sequencing data concerning the nonhomologous junctional fragments are also consistent with the physical mapping carried out on the PCR products (Fig. 5). Third, whereas a junctional fragment characteristic of the homologous product was observed for both sets of cell lines, fragments characteristic of nonhomologous products showed up for cells of one set only. These differences can be readily reconciled with those already observed during analysis of the Hirt extracts themselves by the Southern procedure. However, that analysis had led us to expect some large junctional fragments from R1-4 and Ori-1 cells which PCR did not generate even though other experiments indicate that it could have (16). Such failure suggests that the molecular shorter than pSVI-1 but larger than P155 and detected by blotting in R1-4 and Ori-1 cells may result, not from simple deletions of pSVI-1, but from more complex rearrangements, such as deletions/inversions, rendering the PCR protocol inoperative.

The striking feature of the recombination events which were successfully characterized is the nonrandom joining of sites within the region of pSVI-1 tested. That none of the deletions sequenced had an end point in the Py early coding region was to be expected, if one assumes that HRP quickly disappear from cells when they have not retained the trans-acting functions allowing Py DNA to replicate. However, this hypothesis is unlikely because the viral trans-acting functions are presumably expressed in the same cells by pSVI-1 and/or P155. More straightforward was the observation that not a single HRP could be detected that contained two intact S repeats, with or without intervening sequences between them (Table 1). In contrast, in a number of instances, one or both of the deletion end points were found within or near one S repeat (Table 1).

In an earlier report, we have shown that S repeats are preferred sites for recombination leading to the formation of homologous products (10). Indeed, constructs containing larger homologies that extend the S repeats were found to yield P155 at a higher frequency than RmI itself, but via a crossover still occurring between the S repeats (10). This observation suggests that the first step in recombination is pairing between the homologues, which is then followed by crossover at the preferred site (10). Our data concerning the recombination of pSVI-1 in Ori-1 and R1-4 cells can be readily interpreted in the light of this model. In Fig. 7 we show in various alignments how the two sets of sequences, in which most of the deletion end points are assigned to HRP, are mapped. For each alignment, we indicate the deletions with end points that match one another, with an accuracy of ±150 bp. Several observations emerge from such an analysis. First, few if any HRP were found in which the deletion end points could be matched while the two S repeats were in perfect register. This is what would be expected if a perfect alignment of S repeats favored the generation of the homologous recombinant over that of nonhomologous recombinants. Second, in many instances, one end point fell either within the early S repeat or within 65 bp of it. However, in alignment C for instance, four deletions show matching end points located several hundred bp to the right of the early S repeat. This observation draws the attention to an unusually high concentration of deletion end points between coordinates 3600 and 3900 in the Py late-coding region. HRP with deletions extending further toward the Py Ori in the late-coding region would not have been registered during PCR considering which primers were used (Fig. 3). Judging from the deletions with end points 3600–3900 (Table 1) even those unamplified junctions would have originated in all likelihood from HRP somewhat smaller than P155.

We have assumed previously that recombination at or near the S repeats is dependent not only on some pairing between the two sites to be joined, but also on a process or factor propagated along the DNA from the Py Ori, which would trigger crossover (10, 16). The data presented in Fig. 7 may indicate that in Ori-1 and R1-4 cells, the S repeats are paired in an early step of recombination, but tend not to remain in perfect register. As the process traveling from the Py Ori reaches the early S repeat which appears the more active in recombination because of its position (10), crossover between matched stretches of DNA occurs, as long as some homology exists between them. Hence, P155 or a HRP is formed. This model gains support from our recent observation that reducing either one of the S repeats to less than 182 bp endows a deleted p1-1 (Fig. 2B) with the ability to yield not only P155.

![Fig. 7. Deletion end points in pSVI-1.](image-url)
but also HRP in normal mouse 3T6 cells (16).

It has been proposed that recombination of DNA transfected into mammalian cells starts with the introduction of one or more double-stranded breaks (2, 21). Depending on the nature of the subsequent events, rejoicing of ends would ultimately result in the generation of homologous or nonhomologous recombinants (22). Could S repeats represent privileged sites for recombination because they are particularly susceptible to such double-stranded breaks in the absence of any sort of pairing? This interpretation would not allow us to account for our results unless additional assumptions were made. Whereas the two S repeats in pSVI-1 are identical in sequence, deletion end points are far more frequently located near the early rather than the late repeat. Arguably, this could indicate that the late S repeat is often part of the sequences deleted because double-strand breaking on the late side of the Py Ori occurs predominantly between viral coordinates 3600 and 3900. However, the fact that PI-1 with a partly deleted sequence remote from either S repeat. This brings us back to one final consideration: what makes R1-4 and Ori-1 cells different from 3T6 or SCOP-T1 cells? We have hypothesized that in the SV40-transformed cells the SV40 LT encoded by the resident viral genome and the Py LT encoded previously that in the SV40-transformed cells the SV40 LT encoded by the resident viral genome and the Py LT encoded P155 and HRP does not depend upon the activity of a viral sequence remote from either S repeat. When DNA-bound, such oligomers would confer to the complex formed by the paired repeats an altered topology (9) which would entail the unstable interaction assumed by the model presented here. Interestingly, R1-4 cells synthesize a SV40 "super" T in addition to the SV40 "lytic" T common to both R1-4 and Ori-1 cells (22). As, however, only six nonhomologous junctions were successfully cloned from Ori-1 cells a meaningful comparison between the recombination products of, respectively, R1-4 and Ori-1 cells, could not be carried out at this stage of our work.

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