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Recombination Between Endogenous and Exogenous Simian Virus 40 Genes

II. Biochemical Evidence for Genetic Exchange

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The genome of the simian virus 40 (SV40) temperature-sensitive (*ts*) mutant *tsD202* rescued by passage on transformed permissive monkey lines (see accompanying paper [Y. Gluzman et al., J. Virol. 24:534-540, 1977]) was analyzed by restriction endonuclease cleavage mapping to obtain biochemical evidence that the rescue of the *ts* phenotype results from recombination with the resident SV40 genome of the transformed cell. It was demonstrated that the endonuclease R · *Hae*III cleavage site, which is located at 0.9 map unit in the standard viral genome (and which is in the proximity of the known map position of the *tsD* lesion), is missing in the DNAs of the parental *tsD202* virus and of three independent revertants of *tsD202*. In contrast, this cleavage site was shown to be present in the DNAs of four out of five independently derived rescued *D202* populations and in the DNA of the SV40 strain, 777, used to transform the monkey cells. Comparison of the endonuclease R · *Hin*(II + III) cleavage patterns of SV40 strain 777 DNA and *tsD202* DNA revealed differences in the electrophoretic mobilities of *Hin* fragments A, B, and F. However, the corresponding *Hin* fragments from all four rescued *D202* genomes were identical in their mobilities to those of *tsD202* DNA, indicating that these regions of the rescued *D202* genome are characteristic of the *tsD202* parent. We conclude, therefore, that the genome of the rescued *D202* virus is a true recombinant, since it contains restriction endonuclease cleavage sites characteristic of both parents, the endogenous resident SV40 genome of the transformed monkey cells and the exogenous *tsD202* mutant.

In the accompanying paper (7), we described a system for selecting potential recombinants between the resident endogenous simian virus 40 (SV40) genome of transformed permissive monkey cells and a superinfecting (exogenous) temperature-sensitive (*ts*) SV40 mutant (*tsD202*). We showed that passage, at the permissive temperature, of *tsD202* in each of three lines of transformed permissive monkey cells results in the emergence of temperature-resistant virus whose plating efficiency on nontransformed monkey cells at the restrictive temperature was indistinguishable from that of wild-type SV40. The amount of rescued *tsD202* virus that appeared after passage in transformed cells was 10^3- to 10^6-fold greater than the amount of spontaneously occurring revertant *D202* that appeared after an equal number of passages in nontransformed monkey cells. In the present paper, we present biochemical evidence, based upon restriction endonuclease cleavage analyses of the rescued viral genome, that the repair of the *ts* phenotype is due to recombination with the endogenous resident SV40 genome of the transformed monkey cells.

MATERIALS AND METHODS

Viruses and viral DNA. The SV40 *ts* mutant, revertant, and recombinant populations are described in the preceding publication (7) and in Table 1 and Results in this paper. The wild-type progenitor strain of *tsD202* (which we call WT-M) was kindly provided by R. Martin (2).

To prepare viral DNA, monkey BSC-1 cells were infected at a multiplicity of approximately 2 PFU/cell and incubated at either 33.5 (*tsD202*) or 37°C (all other viruses). For radioactive viral DNA, the infected cultures were labeled at 24 h postinfection with [^{3}H]-thymidine (5 μCi/ml, 24 Ci/mmol) or [^{14}C]thymidine (0.5 μCi/ml, 58 mCi/mmol). At 48 to 72 h postinfection, viral DNA was extracted by the Hirt procedure (9), and the supercoiled SV40 DNA I was purified by ethidium bromide-cesium chloride density gradient centrifugation as described elsewhere (3). In some cases, SV40 DNA I was further purified by sedimentation on 5 to 20% neutral sucrose gradients (3).

Restriction endonucleases. The endonuclease R · *Hin*(II + III) mixture (17) is referred to as *Hin*.

TABLE 1. *History of the recombinant and revertant D202 isolates*

Virus	Derived from passages on (monkey lines):
Recombinant *D202*[a]	
Rec 1	C11
Rec 2	C6
Rec 3	C11
Rec 4	C11
Rec 5	C2
Revertant *D202*[b]	CV1

[a] Rec 1 to 5 are independently derived recombinant *D202* viruses isolated from plaques that appeared at 40.5°C on monkey CV1 monolayers infected with *tsD202* populations that had been previously passaged once (Rec 4) or twice (Rec 1 to 3, Rec 5) at 33.5°C on the indicated transformed monkey lines. Rec 1, Rec 3, and Rec 4 emerged during independent passages of *tsD202* on the C11 line (see Table 3, reference 7). Rec 4 was subjected to an additional cycle of plaque purification on CV1 monolayers at 40.5°C.

[b] Revertant *D202* viruses were isolated from plaques that appeared at 40.5°C on CV1 monolayers infected with *tsD202* passaged twice (at 33.5°C) on monkey CV1 cells (the nontransformed parent of the C2, C6, and C11 lines) (7). Three isolates (each from an independent set of passages on CV1 cells) were made.

Endonuclease R · *Hin*III (17) is designated *Hin*III. Endonuclease R · *Hae*III (17) is abbreviated *Hae*, and endonuclease R · *Eco*RI is called *Eco*RI. *Eco*RI was prepared from *Escherichia coli* strain RY-13 according to Greene et al. (8). *Hin* and *Hae* were purchased from New England BioLabs, Beverly, Mass.

The DNAs were digested with excess restriction endonucleases at 37°C for 4 to 18 h in buffer containing 10 mM Tris-hydrochloride (pH 7.8), 6 mM $MgCl_2$, 6 mM β-mercaptoethanol, and 50 mM NaCl. In the case of *Eco*RI, the NaCl concentration of the digestion buffer was increased to 0.1 M. Reactions were stopped by the addition of EDTA to 10 mM.

Gel electrophoresis. The restriction endonuclease cleavage products were analyzed on 4% polyacrylamide vertical slab gels (22 by 14 by 0.4 cm) in a running buffer containing 40 mM Tris-hydrochloride–20 mM sodium acetate–2 mM EDTA (pH 7.8) as described by Danna et al. (4). Samples containing 0.5 to 1 μg of cleaved DNA in 15% sucrose–0.1% bromophenol blue were applied to each gel slot, and electrophoresis was carried out at 4°C at the voltages and for the times indicated in the figure legends. For visualization of the cleavage products, the gels were stained with ethidium bromide (0.5 μg/ml) and photographed with short-wave UV light (15). When labeled DNA was applied, the gels were cut into strips, corresponding to each slot, which were then sliced into 1- to 2-mm segments. The labeled DNA was extracted from each gel segment by incubation at 65°C for 24 to 48 h in 0.5 ml of 33% (vol/vol) hydrogen peroxide, and the radioactivity was determined in Triton-toluene scintillation fluid (22).

Preparative gel electrophoresis of *Hin*III fragment E (see Results) was carried out on 1.4% cylindrical (16 by 0.7 cm) agarose gels (8 mA/gel, 4 h at 4°C), using a running buffer containing 90 mM Tris-hydrochloride, 90 mM boric acid, and 2.5 mM EDTA (pH 8.2). The gel was stained and visualized under UV light as above. The gel region containing *Hin*III fragment E was excised, and the DNA was recovered by electroelution (11). Ethidium bromide was removed by extraction with isopropanol.

RESULTS

Endogenous, exogenous, and putative recombinant SV40 populations. As described previously (6), the C2, C6, and C11 lines of permissive monkey CV1 cells were transformed by UV-inactivated SV40 strain 777. These transformed cells contain, on the average, one to two SV40 genome equivalents, and most, if not all, of the viral sequences are associated with the cells' chromosomal DNA (6). We refer to the endogenous resident viral genome as SV40 777.

In the preceding publication (7), we showed that passage of SV40 *tsD202* (the superinfecting, exogenous virus) in each of the permissive transformed monkey lines results in the emergence of virus that plates like wild-type SV40 at the restrictive temperature (40.5°C), and we further showed that the rescue of the *tsD202* phenotype is not due to the selection of preexisting revertants or to complementation effects. We refer to the rescued *tsD202* virus as the putative recombinant virus (in short, recombinant *D202* virus). Passage of SV40 *tsD202* on normal CV1 cells generates only minute amounts of virus, which produce plaques at the restrictive temperature (see Table 3, reference 7). Such a rare, spontaneously occurring revertant virus is referred to as revertant *D202*. The history of the five independently derived recombinant *D202* populations, and of the three revertant *D202* populations, is summarized in Table 1.

Evidence for genetic exchange in the region where the *tsD* lesion maps. The rationale for the following experiments can be most readily explained by reference to the *Hin* and *Hae* cleavage maps of SV40 DNA (Fig. 1). The *tsD* lesion is located within *Hin* fragment E at approximately 0.9 map unit from the *Eco*RI site (12, 13, 16). This location is close to the *Hae* cleavage site that defines the junction between the H and I fragments. Below, we will show that this cleavage site is missing in the *tsD202* and revertant genomes and that it is present in both the SV40 777 genome and in four of the five recombinant *D202* genomes. The *Hae* cleavage site, which defines the junction between the H and I fragments, thus serves as a marker for the presence of a segment of the endogenous SV40 777 DNA in the recombinant *D202* genomes.

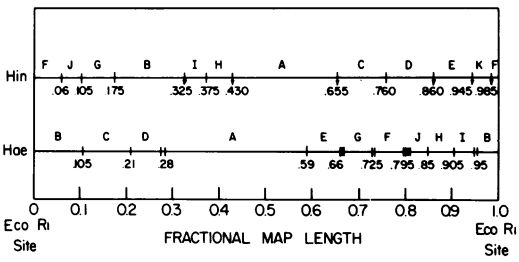

FIG. 1. *Hin and Hae cleavage maps of SV40 DNA. Hin refers to the mixture Hin(II + III); the HinIII cleavage sites are designated by the arrows. Fractional map length refers to the relative distance, in arbitrary map units, from the EcoRI site. Only the major classes of fragments in the Hin and Hae maps are lettered (adapted from Nathans and Smith [14] and Yang et al. [21]).*

The DNAs of SV40 777, *tsD202*, revertant *D202*, and three recombinant *D202* populations (Rec 1 to 3, Table 1) were digested to completion with *Hae*. The cleavage products were then separated by gel electrophoresis as shown in Fig. 2. *Hae* cleaves SV40 DNA into 10 larger classes of fragments (A through J) and a number of smaller classes of fragments. The gel electropherogram of SV40 777 DNA (also referred to as 777 DNA) (slot 6, Fig. 2) shows the 10 major classes, A through J (the minor classes ran off the gel under the conditions of electrophoresis used). *Hae* cleavage of SV40 *tsD202* and revertant *D202* DNA (slots 5 and 4, Fig. 2) generated nine major classes of fragments; two of the expected classes, fragments H and I, are missing, whereas a new class of fragments (designated C′ in Fig. 2) has appeared. These results can be most readily explained on the basis that the *tsD202* and revertant *D202* genomes lack the cleavage site that generates the H and I fragments, and consequently H and I migrate as the single fragment C′. The estimated size of the C′ fragment (~600 base pairs) fits well with the sum of the estimated sizes of the H and I fragments (350 and 240 base pairs, respectively). Additional evidence that the *tsD202* and revertant *D202* genomes lack this specific *Hae* cleavage site will be presented below. The *Hae* cleavage patterns of the three independently derived recombinant *D202* DNAs (slots 1 to 3, Fig. 2) are similar to that of SV40 777 DNA in that the H and I fragments are present and the C′ class of fragments is missing. This indicates that in contrast to *tsD202* and revertant *D202*, the recombinant *D202* genomes contain the *Hae* cleavage site at approximately 0.9 map unit. The *Hae* cleavage patterns of an additional recombinant *D202* population (Rec 4, Table 1) and two other revertant DNA populations were identical to those of the recombinant and revertant DNAs shown in Fig. 2. However, the *Hae* cleavage pattern of the fifth recombinant *D202* population examined (Rec 5, Table 1) was indistinguishable from that of the revertant *D202* DNA (data not shown). It will be noted from Fig. 2 that *Hae* fragment B from 777 DNA (designated B′ in slot 6) migrates faster than *Hae* B from the other DNAs. The reason for the faster migration of *Hae* B from 777 DNA will be clarified later.

To obtain additional evidence for genetic exchange in the region where the *tsD* lesion maps, the experiment described schematically in Fig. 3 was carried out. If the *Hae* site at 0.9 map unit is present, then digestion of the isolated *Hin* E fragment by *Hae* will produce two products, the larger of which should comigrate with *Hae* fragment I (Fig. 3). Accordingly, ^{3}H-labeled *Hin* E fragments were isolated from the DNAs of SV40 777, *tsD202*, revertant *D202*, and recombinant *D202* (Rec 1). Each of the radioactive *Hin* E fragments was then mixed with unlabeled SV40 DNA (as a marker) and cleaved to completion with the *Hae* enzyme. After electrophoresis of the products, the gel was stained with ethidium bromide and photographed to visualize the cleavage pattern of the unlabeled SV40 marker DNA. The gel was then sliced to determine the distribution of ^{3}H radioactivity. The results are shown in Fig. 4, in which a photograph of one of the stained gel strips (the cleavage patterns of the unlabeled SV40 marker DNAs were all identical) has been aligned with the results of the radioactivity measurements. It will be noted from panels 2 and 3 that the *Hin* E fragments derived from the 777 and recombinant *D202* DNAs were, indeed, cleaved by *Hae* into two products, the larger of which migrated in the gel together with *Hae* fragment I (cf. panel 6). In contrast, digestion by *Hae* of the *Hin* E fragments from the *tsD202* and revertant *D202* DNAs produced no change in electrophoretic mobility (panels 1 and 4). The results shown in Fig. 2 and 4 thus establish that whereas the *Hae* cleavage site, which is located at 0.9 map unit in wild-type SV40 777 DNA, is lacking in the *tsD202* and revertant *D202* genomes, it is present in four out of the five recombinant *D202* genomes isolated. Furthermore, this particular *Hae* cleavage site is located close to the known map position of the *tsD* lesion.

Evidence that other regions of the recombinant *D202* genome have not been exchanged. In the previous section, we showed that four independently derived recombinant *D202* genomes contain a segment (defined by the presence of an *Hae* cleavage site) that is characteristic of the resident SV40 genome of the transformed monkey cells. The following

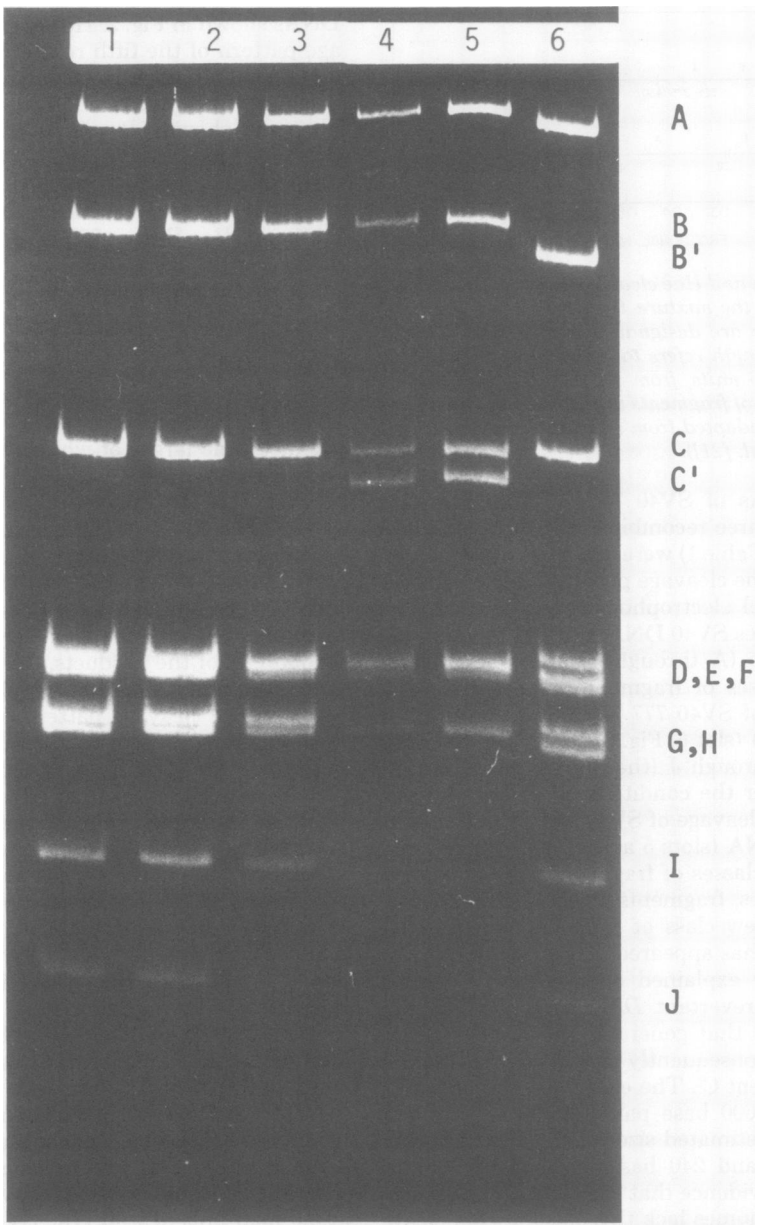

FIG. 2. *Hae digestion of SV40 777, tsD202, and recombinant D202 DNAs. The conditions of digestion and the separation of the products on 4% polyacrylamide gels (9.5 h, 4°C, 200 V) are described in the text. The cleavage patterns of three different recombinant D202 DNA populations (Rec 1 to 3, Table 1) are shown in slots 1 to 3; that of a revertant D202 population is shown in slot 4, and those of the tsD202 and 777 DNAs are shown in slots 5 and 6, respectively. The Hae fragment classes are designated A through J.*

experiments show that other regions of the recombinant *D202* DNA are characterstic of the parental *tsD202* genome and have thus not been exchanged. These experiments exploit differences in the electrophoretic mobilities of *Hin* fragments A, B, and F from SV40 strain 777 and from the wild-type (progenitor) strain of *tsD202*.

*Hin*III cleaves SV40 DNA into six classes of fragments, and the combination of *Hin*(II + III) cleaves the virus DNA into 11 main classes, designated A through K (Fig. 1). The cleavage of SV40 777 and *tsD202* DNAs by these enzymes is shown in Fig. 5A. The *Hin*III cleavage patterns appear to be identical (slots 6 and 1). The *Hin*(II + III) cleavage patterns, however, show

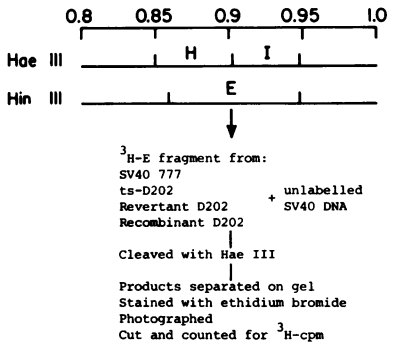

FIG. 3. *Outline of the experiment to determine the presence or absence of the Hae cleavage site within Hin fragment E.*

significant differences around the region of fragments F and E. Band 5 of the *Hin*(II + III) cleavage products from 777 DNA (slot 5) contains the two classes of fragments F and E (the position of fragment E is independently determined by band 5 from the *Hin*III cleavage products shown in slot 6). Slot 3 shows that *Hin*(II + III) cleavage of *tsD202* DNA generates an extra class of fragments that appears between bands 4 and 5 of the 777 digest. These results suggested the possibility that the *Hin* F fragments of 777 and *tsD202* DNAs differ in size. To clarify this point further, the DNAs were digested with *Hin*(II + III) followed by *Eco*RI. *Eco*RI cleaves SV40 DNA at a unique site within F, generating one very small product and one

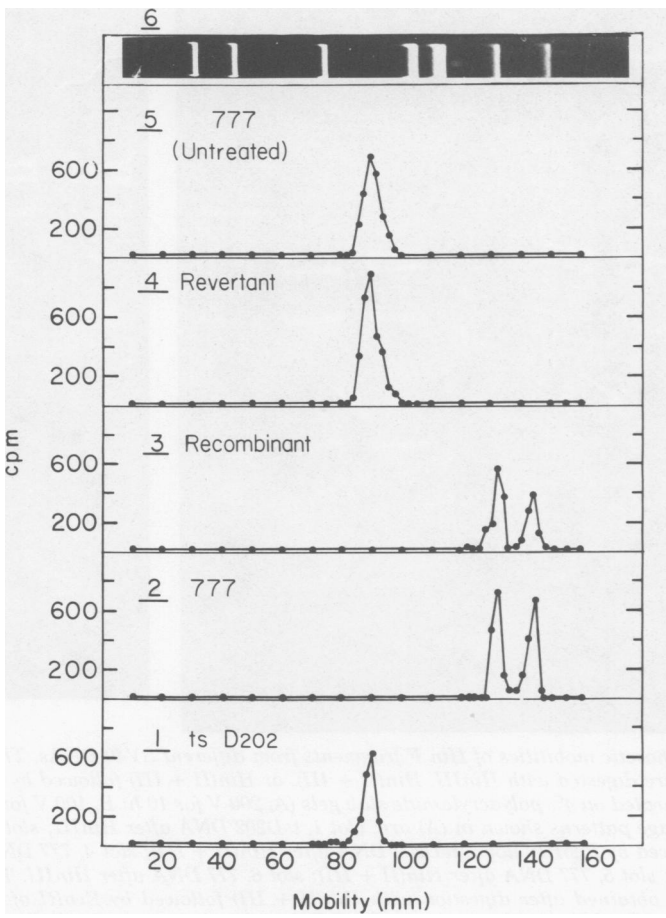

FIG. 4. *Hae digestion of Hin fragment E. ^{3}H-labeled Hin fragment E, isolated from each DNA population by electrophoresis in a 1.4% agarose gel, was digested with Hae in the presence of 1 to 1.5 μg of unlabeled total SV40 DNA (as a marker), and the products were separated on a 4% polyacrylamide slab gel for 9.5 h at 200 V. To visualize the marker DNA digestion products, the gel was stained with ethidium bromide and photographed. The slab gel was then cut into strips (corresponding to each slot), sliced, and counted for ^{3}H radioactivity as described in the text. Panel 6 shows an aligned photograph of one strip of the stained gel in which the major Hae fragment classes can be observed (from left to right: A, B, C, D + E + F, G, H, I, and J). Panels 1 to 4 show the migration of the ^{3}H-labeled Hin E fragments, from the indicated DNAs, after digestion with Hae. Panel 5 shows the results of a control reaction in which the ^{3}H-labeled 777 Hin E fragment was added after the digestion of the marker DNA with Hae had been terminated.*

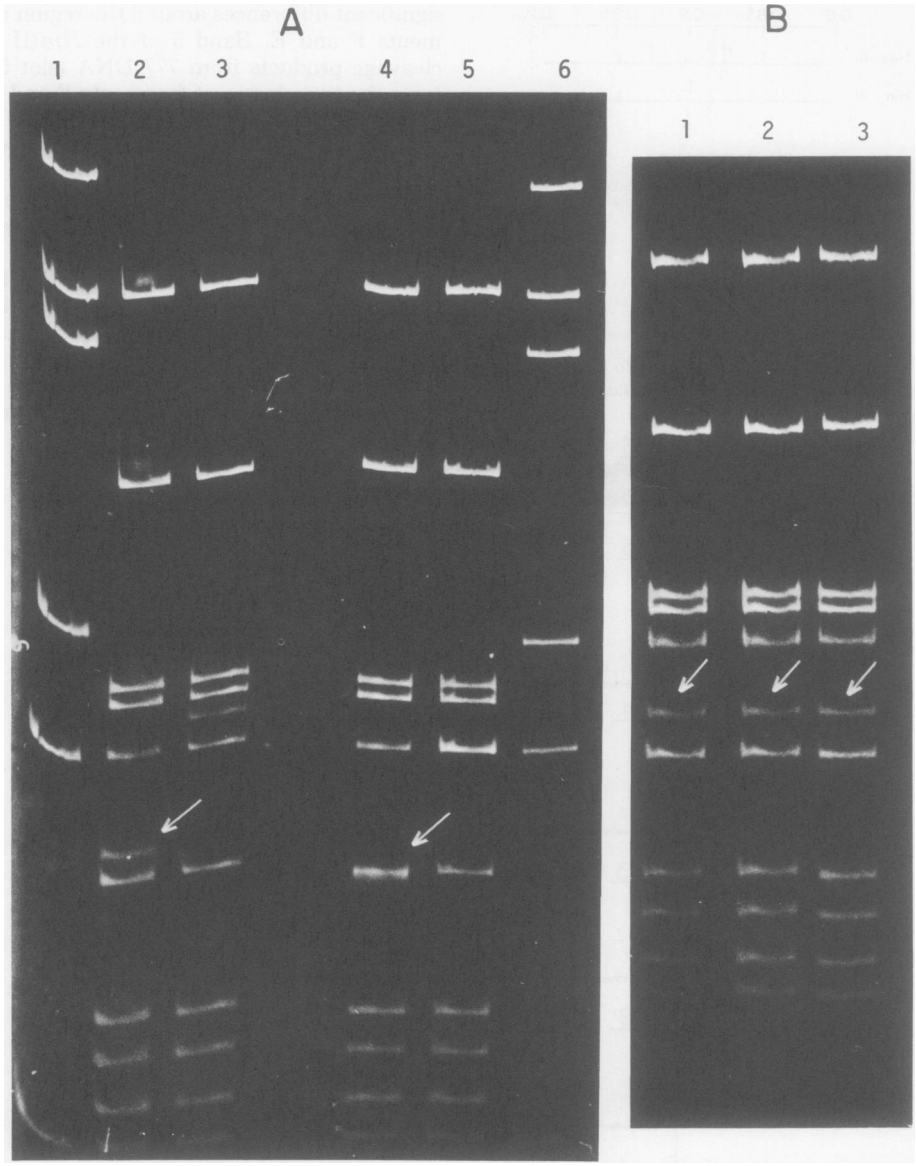

FIG. 5. *Electrophoretic mobilities of Hin F fragments from different SV40 DNAs. The DNA preparations indicated below were digested with HinIII, Hin(II + III), or Hin(II + III) followed by EcoRI. The cleavage products were separated on 4% polyacrylamide slab gels (A, 200 V for 10 h; B, 100 V for 10 h and then 220 V for 2 h). The cleavage patterns shown in (A) are: slot 1, tsD202 DNA after HinIII; slot 2, tsD202 DNA after Hin(II + III) followed by EcoRI: slot 3, tsD202 DNA after Hin(II + III); slot 4, 777 DNA after Hin(II + III) followed by EcoRI; slot 5, 777 DNA after Hin(II + III); slot 6, 777 DNA after HinIII. The cleavage patterns shown in (B) were obtained after digestion with Hin(II + III) followed by EcoRI of revertant D202 DNA (slot 1), recombinant D202 DNA (slot 2), and tsD202 DNA (slot 3). The arrows indicate the larger product obtained when Hin F is digested with EcoRI (see text).*

larger product (Fig. 1). The results of the double digestion are shown in slots 2 and 4 of Fig. 5A. In both cases, digestion by *Eco*RI resulted in the disappearance of the original *Hin* F fragments and in the appearance of new shorter fragments (see arrows), which correspond to the larger segment cleaved from *Hin* F by *Eco*RI (the smaller product ran off the gel under the conditions of electrophoresis used). Clearly, the RI-cleaved F fragment migrates slower in the case of *tsD202* DNA (slot 2) than in the case of 777 DNA (slot 4), where it migrates together

with the *Hin* G fragment. Figure 5B compares the migration of the products generated when the *tsD202,* revertant *D202,* and recombinant *D202* (Rec 1) DNAs were digested with *Hin*(II + III) followed by *Eco*RI. It will be noted that the mobilities of the RI-cleaved F fragments are similar in each case, indicating that the size of the *Hin* F fragment from recombinant *D202* DNA is similar to that from the parental *tsD202* genome and different from that of the 777 genome.

The *Hin* cleavage patterns of the different SV40 DNAs have also revealed differences in the sizes (mobilities) of fragments A and B. Figure 6 compares the cleavage patterns of ^{14}C-labeled 777 DNA with those of ^{3}H-labeled *tsD202* DNA (panel A), ^{3}H-labeled recombinant *D202* DNA (panel B), or ^{3}H-labeled revertant *D202* DNA (panel C). In all three cases, *Hin* fragments A and B from 777 DNA migrate slower (and are therefore presumably larger) than the same fragment classes cleaved from the other DNAs. *Hin* fragments A and B from *tsD202,* recombinant *D202,* and revertant *D202* all appear to have the same electrophoretic mobility. The differences in the mobilities of the RI-cleaved *Hin* F fragments can also be seen in Fig. 6. In each case, the RI-cleaved *Hin* F fragment from *tsD202,* recombinant *D202,* or revertant *D202* (designated by the arrows) migrates slower than the RI-cleaved *Hin* F fragment from 777 DNA (which comigrates with *Hin* G).

Reference to the SV40 cleavage map in Fig. 1 will show that differences in the size of *Hin* fragment B will alter the size of *Hae* fragment D, and differences in the size of *Hin* F will be reflected in the size of *Hae* fragment B. The *Hae* cleavage patterns have confirmed that fragment D from 777 DNA migrates slower than fragment D from the *tsD202,* revertant *D202,* or recombinant *D202* DNA (data not shown), and that fragment B from 777 DNA migrates faster than that from *tsD202,* revertant, or recombinant DNA (see B′ in slot 6 of Fig. 2). We therefore conclude that the regions of the recombinant *D202* genome defined by *Hin* fragments A, B, and F (or the overlapping *Hae* fragments) are characteristic of the *tsD202* parental genome rather than the endogenous 777 genome of the transformed monkey lines. The biochemical markers characteristic of the parental (endogenous 777 and exogenous *tsD202*) revertant *D202* and recombinant *D202* genomes are summarized in Table 2.

DISCUSSION

As described in the accompanying paper (7), the SV40 recombinant *D202* genomes emerged during passage of SV40 *tsD202* at the permissive temperature in permissive transformed monkey cells whose resident SV40 777 genome is wild type with respect to the *tsD* lesion. The recombinant ("rescued") virus was isolated from plaques produced by infecting normal monkey cells at the restrictive temperature with the passaged *ts* population. The objective of the studies described herein was to obtain biochemical evidence for the genetic exchange, using restriction endonuclease cleavage sites as markers for the presence of segments from both parents, the endogenous resident SV40 777 genome of the transformed cell and the superinfecting, exogenous *tsD202* genome. It is important to stress that each of the recombinant *D202* genomes analyzed arose during independent sets of passages of *tsD202,* either in the same or a different line of permissive transformed monkey cells (see Table 1). The three revertant *D202* populations examined arose during independent passages of *tsD202* on normal monkey cells.

The proximity of the *Hae*III cleavage site, located at about 0.9 map unit in the standard SV40 genome, to the known map position of the *tsD* lesion (12, 13, 16) made this restriction endonuclease an obvious candidate for the biochemical marker system being sought. Indeed, we have demonstrated that whereas this *Hae* site is missing in the parental *tsD202* genome and in each of the three independent revertant genomes of *tsD202,* it is present in the DNA of four out of the five independent recombinant *D202* populations examined. Since the *Hae* site at 0.9 map unit is also present in the DNA of SV40 777 used to transform the monkey cells, it serves as one marker for genetic exchange between *tsD202* and the resident viral genome of the transformed cells. The reason for the absence of this marker in the fifth recombinant *D202* population (Rec 5) is unknown. Several possibilities, however, may be considered. Since the *Hae* site under discussion may be located a few base pairs distant from the corresponding position of the *tsD* lesion, Rec 5 may represent a minor class of recombinants in which the segment derived from the resident SV40 genome does not contain the *Hae* site. Alternatively, the cleavage recognition sequence may have undergone alteration during the recombination process. A third possibility is that Rec 5 is a true revertant of *tsD202,* though this seems unlikely on statistical grounds since the reversion frequency of *tsD202* is extremely low (see Table 3, reference 7). Additional cleavage maps of Rec 5 DNA will be required to distinguish between these alternatives.

The second biochemical marker system used was based upon differences between the SV40

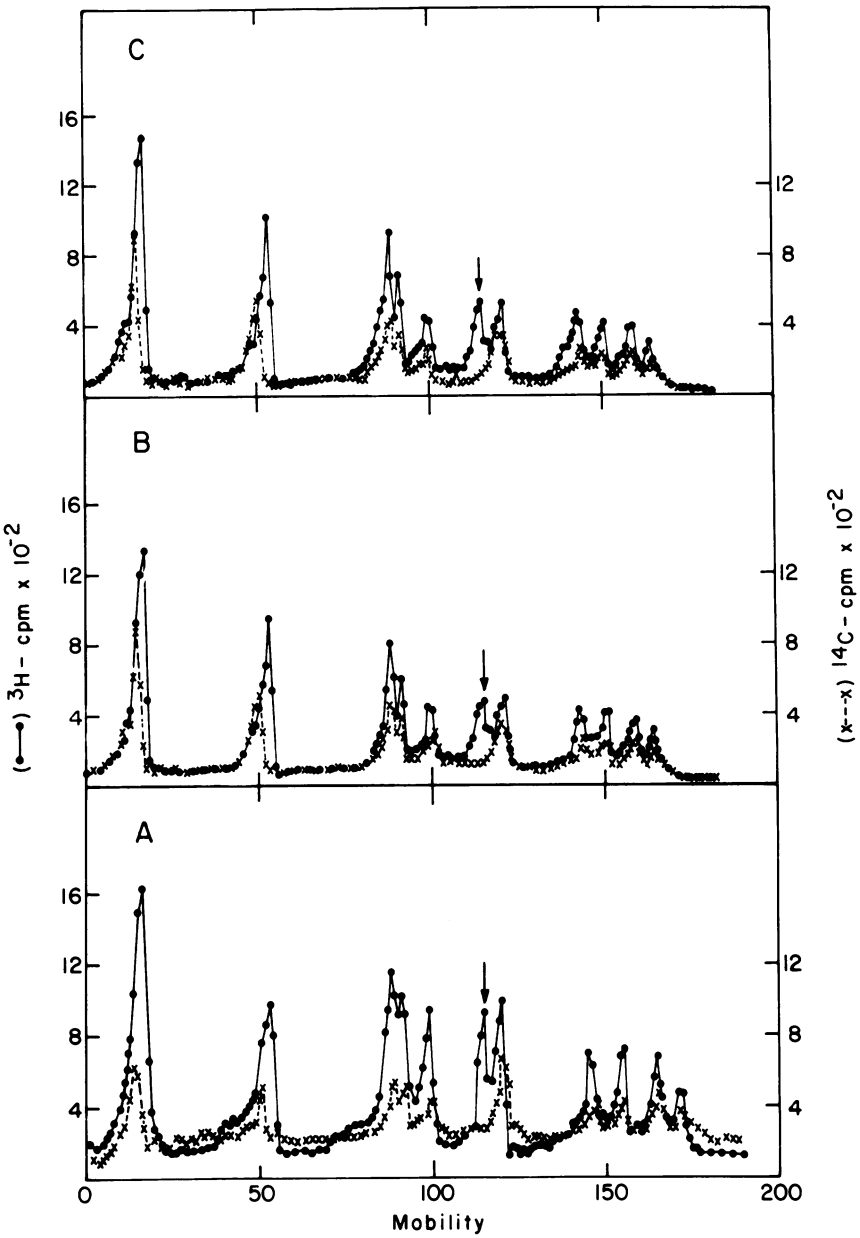

FIG. 6. *Electrophoretic mobilities of Hin fragments A and B from different SV40 DNAs.* ^{14}*C-labeled* (×) *SV40 777 DNA was mixed with* ^{3}*H-labeled* (●) *tsD202 DNA (A), recombinant D202 DNA (B), or revertant D202 DNA (C) and digested with Hin(II + III) followed by EcoRI, as described in the legend to Fig. 5. The cleavage products were separated by electrophoresis on a 4% polyacrylamide slab gel (100 V for 18 h). The gel was sliced, and the distribution of* ^{3}*H- and* ^{14}*C-labeled material was determined as described in the text. The arrows indicate the larger segment cleaved from Hin F after digestion with EcoRI (see text).*

777 and the progenitor strain (WT-M) of *tsD202* in respect to the *Hin* cleavage patterns. We have shown that *Hin* A and *Hin* B from 777 DNA migrate slower than the corresponding fragments from *tsD202* DNA, and 777 *Hin* F migrates faster than *Hin* F from *tsD202* DNA. These differences are also reflected in the migration of the overlapping *Hae* fragments and therefore arise from deletions (or insertions) rather than from single base changes within the cleavage recognition sequences. In the case of *Hin* F, which is part of the coding sequence for

TABLE 2. *Comparison of the parental, revertant, and recombinant SV40 genomes*

Virus	Presence of *Hae* site at ca. 0.9 map unit	Mobility of Hin fragments[a]		
		A	B	F
777[b]	+	L	L	H
WT-M[c]	+	H	H	L
tsD202[d]	−	H	H	L
Revertant *D202*[e]	−	H	H	L
Recombinant *D202*[f]	+	H	H	L

[a] L and H refer, respectively, to a lower and higher mobility of the indicated *Hin* fragments when 777 DNA is compared with the other DNAs. We assume that higher mobility indicates a shorter fragment and lower mobility indicates a larger fragment.

[b] Strain of the resident SV40 genome in the permissive transformed monkey lines C2, C6, and C11 (6).

[c] Progenitor strain of the *ts* mutant *tsD202*.

[d] The parental *ts* mutant that gave rise to the revertant *D202* and recombinant *D202* isolates after passage, respectively, on nontransformed CV1 or transformed C2, C6, and C11 lines (7).

[e] The *Hae* and *Hin* cleavage patterns of each of the three revertant *D202* populations isolated (Table 1) were identical.

[f] Four of the five independently derived recombinant *D202* DNA populations (Rec 1 to 4, Table 1) contained the cleavage markers described above. As noted in the text, the *Hae* site was not present in the Rec 5 DNA population.

the major SV40 capsid protein VP1 (19), the latter conclusion is supported by the observation that the WT-M VP1 polypeptide is larger (by about 3,000 daltons) than that of strain 777 (T. Vogel, unpublished data). The *Hin* A, B, and F fragments cleaved from the recombinant DNAs were all identical in their electrophoretic mobility to those derived from *tsD202* DNA. Hence, we conclude that the regions of the recombinant *D202* genome delineated by *Hin* A, B, and F (0.430 to 0.655, 0.175 to 0.325, and 0.985 to 0.06 map units, respectively) are characteristic of the *tsD202* parent rather than of the 777 parent.

The restriction endonuclease cleavage mapping studies performed so far are not sufficiently comprehensive to define the precise boundaries of the segments exchanged in the four recombinant *D202* populations. However, since all four populations contain the *Hae* site at 0.9 map unit and the *Hin* F fragment derived from all four populations is characteristic of the *tsD202* parent, it appears that the segment exchanged starts somewhere to the left of the *Hae* site and ends at some point before the cleavage site that generates *Hin* fragment F (see Fig. 1). With regard to the region to the left (5′ end) of the *Hae* site, analyses of the *Hin*(II + III) cleavage patterns have failed to reveal any differences in the size of *Hin* fragment D between the recombinant *D202* genomes and that of the *tsD202* parent (T. Vogel, unpublished observations). The different recombinant *D202* populations, however, show some variation in the size of *Hin* fragments E and K; *Hin* fragment E in three of the five populations was found to be slightly larger than that of *tsD202* DNA, and *Hin* fragment K in one recombinant population was observed to be slightly smaller than the corresponding fragment of *tsD202* (T. Vogel, unpublished observations). These studies are being continued to clarify the mechanism of recombination involved.

In considering the types of viable recombinant *D202* genomes that could arise, it is important to take into account that both the nature of the resident SV40 777 genome in the transformed monkey cells and the recombinant isolation procedure will discriminate against certain types of recombinants. For example, the SV40 strain 777 used to transform the monkey cells is a naturally occurring late *ts* mutant (7). The location of this late *ts* lesion is expected to lie somewhere within *Hin* fragments K, F, J, or G (12, 13). Thus, any exchange between *tsD202* and the *Hin* K-G region of the resident SV40 genome will produce a recombinant with a reduced chance of survival during plaque purification at the restrictive temperature. Furthermore, if, as we have suggested previously (6), the *A* gene function of the resident SV40 genome is defective with respect to the initiation of viral DNA synthesis (the *ts-A* class of SV40 mutants do not grow at the restrictive temperature on the transformed permissive monkey lines), then recombinant *D202* genomes containing part of the resident genome corresponding to *Hin* fragment A, H, I, or B (which comprise the early SV40 region) may well be nonviable. Our finding that the *Hin* A and *Hin* B fragments derived from the four recombinant *D202* genomes were characteristic of the *tsD202* parent rather than of the 777 parent is consistent with this notion.

As noted in the accompanying paper (7), the recombinant *D202* virus grows faster than the parental *tsD202* at the permissive temperature. Consequently, the proportion of recombinants is strongly amplified during passage. We cannot, therefore, estimate the frequency with which the original recombination event occurs. It is quite conceivable that the high proportion of recombinant *D202* viruses found in the passage 2 yields of transformed monkey cells (7) represent the amplified progeny of a relatively rare recombination event. Dubbs et al. (5) have described recombination between late SV40 *ts* mutants during mixed infections and have estimated the recombination frequency to be about 2.0×10^{-4}. If it proves possible to obtain reliable estimates of recombination frequency in the system we have described in this and the accompanying paper, then the classical marker-rescue technique used for determining prophage gene order in lysogenic bacteria (1) may be applicable

to the integrated SV40 genome of transformed cells.

Recombination between endogenous integrated viral genes and exogenous viruses is one possible mechanism that can account for the observed heterogeneity in the properties of RNA tumor viruses (18, 20). The generality of this mechanism is extended by our demonstration that such recombination events also occur with a DNA tumor virus. The SV40 sequences found in the genomes of several representatives of the ubiquitous human papovaviruses (10) may be the consequence of a similar mechanism. Indeed, exogenous-endogenous viral recombination events may play a highly significant role in the evolution of persistent viruses in nature.

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