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Genetics

Indiscriminate recombination in simian virus 40-infected monkey cells

(DNA cotransfection/simian virus 40–bacterial virus φX174 hybrid/*in situ* plaque hybridization)

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ABSTRACT **DNA transfection of African green monkey BSC-1 cells with simian virus 40 (SV40) DNA and bacterial virus φX174 replicative form DNA ("cotransfection") yielded stocks containing SV40/φX174 recombinant virus, which was detected by an infectious-center *in situ* plaque hybridization procedure and which was sensitive to anti-SV40 antiserum. The recombinant virus replicated during serial passage. Restriction endonuclease cleavage of the SV40/φX174 DNA indicated that several different types of recombinant DNA structures had arisen. Similar SV40 DNA cotransfection experiments with polyoma virus DNA, bacterial plasmid (pBR322) DNA, and a plasmid-cloned segment of the mouse genome (coding for intracisternal type A particles) yielded stocks that generated recombinant plaques as judged by *in situ* plaque hybridization with the appropriate labeled probe. It appears, therefore, that an active indiscriminate recombination process, incapable of distinguishing between diverse DNAs of prokaryotic and eukaryotic origin, occurs in SV40-infected monkey cells.**

Simian virus 40 (SV40)-infected monkey cells exhibit a range of recombination activities, encompassing the insertion of host cell DNA into the viral genome (ref. 1 and reviewed in refs. 2–4), rearrangements within the viral genome (2, 3), the insertion of SV40 sequences into the adenovirus genome (5) and vice versa (6), and genetic exchange with the endogenous viral genome in SV40-transformed permissive monkey cells (7, 8). With the development of highly sensitive *in situ* plaque hybridization procedures for the detection of hybrid animal virus genomes (9, 10) it seems opportune to examine the possibility that SV40 DNA may recombine with any DNA, of either prokaryotic or eukaryotic origin, that happens to be present in the infected cell. In this report, we show that cotransfection of monkey cells with SV40 DNA and bacterial virus DNA [φX174 replicative form 1 (RF1)] gives rise to recombinant progeny. Using the same approach of DNA cotransfection, we also report strong circumstantial evidence for *in vivo* recombination between SV40 and polyoma virus DNAs, SV40 and bacterial plasmid DNAs, and SV40 and cloned mouse DNA.

MATERIALS AND METHODS

DNA and Cells. φX174 RF1 DNA, plasmid pBR322 DNA, and plasmid pMIA1 DNA (11) were kind gifts of S. Eisenberg, D. Roufa, and E. L. Kuff, respectively. SV40 (strain 777) and polyoma virus (strain IL 11) DNA were isolated and purified by equilibrium centrifugation on cesium chloride density gradients supplemented with ethidium bromide as described (12). Supercoiled DNA was isolated from African green monkey BSC-1 cells infected with recombinant viral stocks by the Hirt procedure (13) at 50 hr after infection and purified as above. Cells were grown and maintained in Dulbecco's modified Eagle's medium with 10% calf serum.

Transfection, Harvest of Virus, and Serial Passage. BSC-1 cells were transfected with DNA by the DEAE-dextran (300 μg/ml) procedure (14), and the resulting viral stocks were harvested by subjecting the lysed cells and medium to sonic vibration. Stocks were passed in series by infecting BSC-1 cells on 9-cm-diameter plates with 1 ml of the previous passage diluted 1:3 in medium.

The Infectious-Center *in Situ* Plaque Hybridization Procedure. Recombinant virus was detected by the *in situ* plaque hybridization technique (9), modified as an infectious center procedure (10). BSC-1 cells were infected with the viral stocks either undiluted or diluted 1:3 in medium, or diluted 1:10 in wild-type SV40 (10^8 plaque-forming units/ml) as specified in Table 1. Five hours after infection, the infected cells were dispersed with trypsin/EDTA; 50,000–200,000 infected cells were then mixed with 3×10^6 uninfected BSC-1 cells, plated in 9-cm dishes, and overlaid with nutrient-agar (0.9% agar and 10% calf serum in Dulbecco's modified Eagle's medium) when monolayer formation was complete. After 5 days of incubation (or as specified) the agar was removed and stored at 4°C. A dry nitrocellulose filter (Schleicher & Schuell, 0.45-μm pore diameter, 8-cm diameter) was then placed on top of the bared cell monolayer. After moistening with saline (0.15 M NaCl/0.01 M Tris·HCl, pH 7.0) the filter with the cell monolayer stuck to its underside was peeled off and placed, cell-side up, for 1 min on top of Whatman 3 MM paper saturated with 0.5 M NaOH containing 1.5 M NaCl. The filter was then blotted dry and the alkali denaturation step, with intermittent drying, was repeated twice more. Finally, the filter was neutralized by three 1-min contacts with Whatman paper saturated with 1 M Tris·HCl (pH 7.0) and 2 × NaCl/Cit (1 × NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate, pH 7.4), dried at room temperature, and baked at 80°C for 3–6 hr.

The filters were preincubated at 67°C in Denhardt's buffer (15) with 6 × NaCl/Cit for 4 hr. The buffer was then removed and replaced with fresh Denhardt's buffer containing 6 × NaCl/Cit, denatured sonicated salmon sperm DNA at 100 μg/ml, and the appropriate [^{32}P]DNA probe [labeled *in vitro* by nick-translation (16); 5×10^7 to 10^8 cpm/μg] at 20,000–40,000 cpm/ml, and incubation was continued at 67°C for a further 18–20 hr. After hybridization, the filters were washed by incubation at 67°C for 1 hr with, successively, 6 × NaCl/Cit, 2 × NaCl/Cit containing 0.1 M $NaPO_4$ buffer (pH 6.5), and 2 × NaCl/Cit alone. Autoradiograms of the filters were made with Agfa Curix x-ray film in conjunction with intensifying screens at −70°C for the time periods specified in the legends. Control filters made from uninfected BSC-1 cells were included in each experiment and displayed no autoradiographic response

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Abbreviations: SV40, simian virus 40; RF1, replicative form 1 (covalently closed circular); NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate, pH 7.4 (standard saline citrate); kb, kilobase.

with any of the [^{32}P]DNA probes used. Recombinant virus was picked from the agar overlay by aligning autoradiographic plaques and agar, using a system of orientation marks described elsewhere (9).

Restriction Endonuclease Digestion, Gel Electrophoresis, and Blot Hybridization. DNA was digested with the various restriction enzymes under the conditions specified by the manufacturer (New England BioLabs), and the products were separated by electrophoresis [40 V for 20 hr in Tris/acetate buffer (8) on 1% agarose slab gels (28 × 10 × 0.5 cm)]. The gel was stained with ethidium bromide (0.5 μg/ml) and photographed under UV light. The products were transferred to nitrocellulose sheets (Schleicher & Schuell) by the Southern technique (17), and the blots were hybridized as described above for *in situ* plaque hybridization except that the concentration of [^{32}P]DNA probe was increased to 300,000 cpm/ml, the time of hybridization at 67°C was increased to 37 hr, and the 6 × NaCl/Cit and 2 × NaCl/Cit/$NaPO_4$ washing buffers contained 0.5% sodium dodecyl sulfate. Conditions of autoradiography are specified in the figure legends.

RESULTS

The protocol was divided into two stages. In stage 1, BSC-1 cells were cotransfected with SV40 DNA and the other DNAs described in Table 1. When typical SV40 cytopathic effects appeared, 9–11 days after infection, the total virus yield of the cotransfected culture was harvested (designated stock A). In stage 2, stock A or its serially passaged derivatives (stocks B–D) were analyzed for the presence of putative recombinant virus by the infectious-center *in situ* plaque hybridization technique, using the appropriate [^{32}P]DNA probe. We have assumed that any SV40 recombinant that arose in the cotransfected culture will be defective and capable of replicating only in cells supporting the multiplication of wild-type virus. The yield of wild-type SV40 virions produced by the DNA-cotransfected cultures was generally sufficient to infect at least 50% of the cells in the infectious-center assay of stage 2; but as an additional precaution, most of the stocks and all of their serially passaged derivatives were diluted 1:10 in excess wild-type SV40 (10^8 plaque-forming units/ml) prior to titration by the infectious-center assay.

Cotransfection with SV40 DNA and φX174 RF1 DNA

The analysis of the viral populations derived from cotransfection with SV40 DNA and φX174 RF1 DNA is shown in Table 1 (Exp. 59) and in Fig. 1. The appearance of foci that reacted with the φX174 [^{32}P]DNA probe depended upon the time of transfer to the nitrocellulose filter. When the transfer was made at 1 and 2 days after infection, no foci were visible. Small indistinct foci first became visible at 3 days after infection (Fig. 1*A*) and these substantially increased in size by 5 days after infection (Fig. 1 *B* and *C*). When stock A was incubated with anti-SV40 antiserum, prior to infection of cells for *in situ* plaque hybridization, no infectious centers that reacted with φX174 [^{32}P]DNA appeared. No foci containing φX174 DNA sequences were detected when cells were "infected" with a 10-day lysate of BSC-1 culture transfected with φX174 DNA in the absence of SV40 DNA.

The putative SV40/φX174 recombinant virus replicated during serial passage and could be isolated from the agar overlay of the *in situ* plaque hybridization assay plates. Fig. 1*D* shows the autoradiographic response obtained when a portion of the agar overlay corresponding to a plaque in Fig. 1*C* was sampled (9), grown to a stock and retitered by the infectious-center assay. Fig. 1 *E* and *F* shows the foci obtained when 1:10 dilutions of the yield of the cotransfected culture (stock A) and

Table 1. Recombinant virus populations derived from DNA cotransfection of BSC-1 cells

Transfection with					*In situ* plaque hybridization (infectious-center assay)*			
Exp.	DNA	Conc., μg/ml	DNA†	Conc., μg/ml	Stock	Dilution, fold	[^{32}P]DNA probe	Autoradiographic plaques/10^6 infected cells
59	SV40	5	φX174 RF1	5	A	3	φX174 RF1	714
					A	3		0‡
					A	10		1133
					B	10		1749
					D	10		3300
62	SV40	1	Polyoma virus	1	A	—	Polyoma	225
	SV40	1	Polyoma virus	5	A	—	virus	675
			Polyoma virus	5	A	—		0
49	SV40	5	pBR322	5	A	—	pBR322	93
			pBR322	5	A	—		0
53	SV40	2	pMIA1	4	A	10	pBR/A	110
			pMIA1	4	A	—		0
32	SV40	5	pBR/A	5	A	10	pBR/A	107
					B	10		250
			pBR/A	5	A	—		0
					A	10		0

* Stock A is the 9- to 11-day virus yield of the DNA-transfected culture; stocks B and D are, respectively, first and third serial passages of stock A. Where indicated, the stocks were diluted 1:3 in medium or 1:10 in medium supplemented with 10^8 plaque-forming units/ml of wild-type SV40. The number of autoradiographic plaques is derived from the average of five replicate plates (each seeded with 50,000–200,000 infected cells and 3 × 10^6 uninfected cells) normalized to 10^6 infected cells.

† pMIA1 (Exp. 53) is a 9.6-kilobase (kb) pBR322 recombinant carrying a 5.3-kb insert of mouse genomic DNA that codes for intracisternal type A retroviruses (11). pBR/A DNA (Exp. 32) is derived by cleavage of pMIA1 with *Hin*dIII and *Eco*RI, generating the 5.3-kb linear mouse DNA insert and a 4.3-kb linear molecule of pBR322 DNA. Seventy percent of the pBR322 DNA was removed by agarose gel electrophoresis (the pBR/A DNA was kindly supplied by E. L. Kuff).

‡ Stock A was incubated with an equal volume of anti-SV40 antiserum prior to the assay. The same virus treated with control serum gave the expected number of autoradiographic plaques.

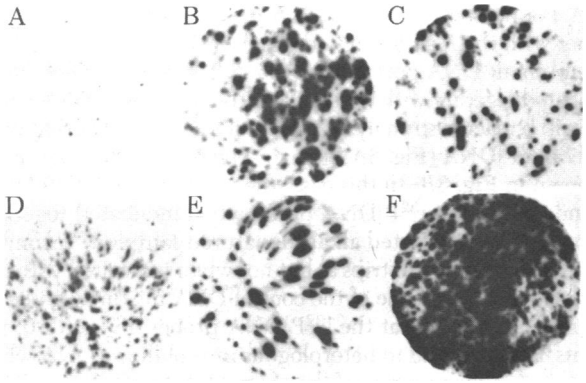

FIG. 1. Autoradiograms showing SV40/ϕX174 recombinant plaques detected the infectious-center *in situ* plaque hybridization procedure. Between 70,000 and 200,000 BSC-1 cells, infected with either stock A or its derivatives (Exp. 59, Table 1) were mixed with 3×10^6 uninfected BSC-1 cells, plated to form a monolayer, overlaid with agar, transferred to nitrocellulose filters at the time intervals noted below, and hybridized with ϕX174 [^{32}P]DNA (5×10^7 cpm/μg, 40,000 cpm/ml). (*A*) 200,000 infected cells, 3-day transfer; (*B*) 200,000 infected cells, 5-day transfer; (*C*) 70,000 infected cells, 5-day transfer. (*D*) Foci obtained (200,000 infected cells, 5-day transfer) when cells were infected with the progeny of a single plaque picked from the agar of the plate shown in *C*. (*E* and *F*) Foci obtained (100,000 infected cells, 5-day transfer) when cells were infected, respectively, with 1:10 dilutions of stocks A and D of Exp. 59.

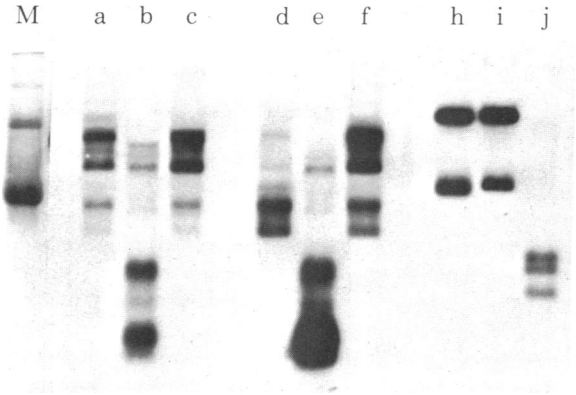

FIG. 2. Blot hybridization of the ϕX174 DNA-containing fragments generated by *Bgl* I and *Hga* I cleavage. The hybridization patterns of stock B (Exp. 59, Table 1) progeny DNA are shown in tracks a (undigested sample), b (*Bgl* I digested), and c (*Hga* I digested). Stock C (Exp. 59, Table 1) progeny DNA patterns are shown in tracks d (undigested sample), e (*Bgl* I digested), and f (*Hga* I digested). Control DNA (authentic ϕX174 RF1 DNA mixed 1:1000 with authentic SV40 DNA) patterns are shown in tracks h (undigested sample), i (*Bgl* I digested), and j (*Hga* I digested). Two micrograms of DNA were electrophoresed in each gel slot and blot-hybridized as described in the text. Tracks a–j were hybridized with ϕX174 [^{32}P]DNA (4.5×10^7 cpm/μg; 300,000 cpm/ml for 37 hr at 67°C; autoradiographic exposure, 4 hr at −70°C). Track M shows an undigested sample of authentic SV40 DNA forms I and II, electrophoresed in the same gel but hybridized with SV40 [^{32}P]DNA at 5000 cpm/ml.

a third consecutive serial passage of that yield (stock D) were titrated. The numbers of foci obtained, out of 10^6 infected cells plated, are summarized in the final column of Table 1, Exp. 59. Stock A diluted 1:10 in an excess of wild-type SV40 produced more ϕX174 DNA-containing infectious centers than the same stock diluted 1:3 in medium. This unexpected increase may be due to the dilution of defective interfering virus, because we have consistently observed that the viral yields of cells transfected with SV40 DNA contain a surprisingly high proportion of rearranged SV40 genomes, as judged by anomalous restriction endonuclease patterns. From the number of ϕX174 autoradiographic plaques produced by stock D (diluted 1:10 in excess wild-type SV40 to optimize the response) and from the titration of stock D for wild-type SV40 infectious centers (by *in situ* plaque hybridization using an SV40 ^{32}P-labeled probe), we estimate that the proportion of putative SV40/ϕX174 recombinants is 2% of the wild-type SV40 in this stock.

Restriction endonuclease cleavage and the evidence for linkage between SV40 and ϕX174 DNA sequences

To obtain information on the possible variety of recombinant structures, the supercoiled progeny DNAs of stocks B and C (Exp. 59) were purified on cesium chloride/ethidium bromide density gradients and digested with *Bgl* I or *Hga* I. The products were separated on a 1% agarose gel, transferred to nitrocellulose (17), and hybridized with ^{32}P-labeled ϕX174 RF1 DNA. *Bgl* I was chosen because it cleaves wild-type SV40 once, close to the replication origin, and does not cleave authentic ϕX174 RF1 DNA (18); *Hga* I cleaves authentic ϕX174 RF1 DNA into 14 fragments but does not cleave wild-type SV40 DNA (18).

The general conclusion of the blot-hybridization results in Fig. 2 is that the ϕX174 sequences are associated with several different classes of DNA molecules. In the undigested DNA (tracks a and d) the sequences were associated with molecules that did not comigrate with components I (supercoiled) and II (relaxed form) of wild-type SV40 DNA (track M) or authentic ϕX174 RF1 DNA (track h). Most of the ϕX174 sequences in the undigested progeny DNA of stock C (track d) were located in two major classes of molecules, both of which migrated faster than supercoiled wild-type SV40 DNA. We assume that the slower-migrating forms that are prominent in track a (and also visible in track d) are the relaxed forms of these two major classes of supercoiled molecules containing ϕX174 sequences. *Bgl* I digestion generated a number of products containing ϕX174 sequences (tracks b and e) and did not, as expected, alter the mobility of authentic ϕX174 RF1 DNA (track i). That most of the ϕX174 sequences were found in several smaller classes of fragments after *Bgl* I digestion (cf. tracks a and b; tracks d and e) suggests that the recombinant structures possess multiple *Bgl* I sites, because single cleavage sites would be expected to convert the supercoiled molecules into slower-migrating unit-length linear forms. The pattern of *Hga* I cleavage is complex, and differences in the cleavage patterns of stock A progeny DNA and the progeny DNAs of stocks B and C were observed. *Hga* I digestion of the progeny DNAs of stocks B and C (Fig. 2, tracks c and f) generated some slower-migrating structures, but it is apparent that not all the molecules were cleaved. The *Hga* I cleavage pattern of stock A progeny DNA revealed, in addition, some products that migrated faster than the undigested sample (data not shown). The digestion of authentic ϕX174 RF1 DNA by *Hga* I is shown in track j. Only the larger classes of fragments are visible; the expected smaller classes either ran off the gel or were lost upon transfer. *Hga* I digestion did not alter the mobility of wild-type SV40 DNA (data not shown).

The progeny DNA of stock A (Exp. 59) was also analyzed by using (in addition to *Bgl* I and *Hga* I) digestion with *Hin*dIII and *Mbo* I, which cleave SV40 DNA but not authentic ϕX174 RF1 DNA (18). The *Bgl* I cleavage pattern was similar to that shown in Fig. 2. The *Hin*dIII and *Mbo* I cleavage patterns confirmed that all of the ϕX174 sequences are associated with DNA molecules containing restriction sites not present in authentic ϕX174 RF1 DNA.

The linkage between the SV40 and ϕX174 DNA sequences

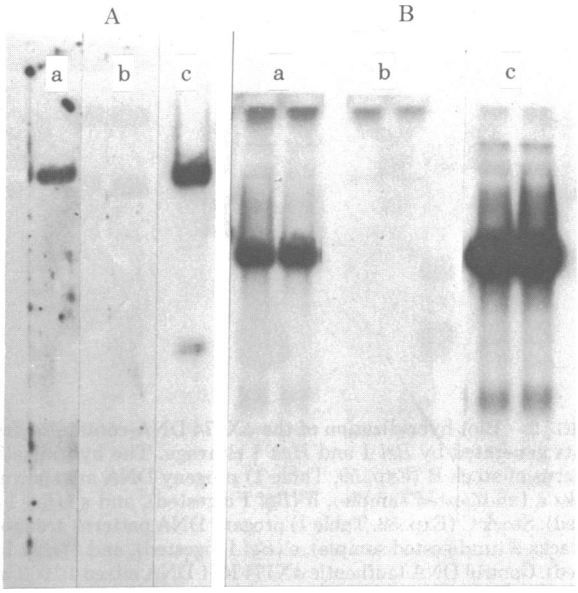

FIG. 3. Linkage between the SV40 and ϕX174 DNA sequences. (*A*) One-microgram samples of linear (*Pst* I digested) ϕX174 RF1 DNA were electrophoresed on a 1% agarose gel and transferred to nitrocellulose. The nitrocellulose blot was cut into strips and hybridized for 23 hr at 67°C in the following mixtures, per ml: strip a, 1.3 μg of *Hga* I-digested and alkali-denatured SV40/ϕX174 recombinant DNA (same DNA as that in Fig. 2, tracks a–c) supplemented with 10^6 cpm of denatured SV40 [^{32}P]DNA (6×10^7 cpm/μg); strip b, 1.3 μg of *Hga* I-digested and alkali-denatured control DNA (1% authentic ϕX174 RF1 DNA and 99% SV40 DNA) supplemented with SV40 [^{32}P]DNA as in a. After autoradiography for 65 hr at −70°C, strip b was rehybridized with heat-denatured ϕX174 [^{32}P]DNA (10,000 cpm/ml; 3.8×10^7 cpm/μg) and reautoradiographed for 17 hr at room temperature (strip c). (*B*) One-microgram samples of linear (*Eco*RI digested) SV40 DNA were electrophoresed on a 1% agarose gel and transferred to nitrocellulose. The nitrocellulose blot was cut into strips (containing duplicate tracks) and hybridized for 22 hr at 67°C in the following, per ml: strip a, 1.3 μg of *Hga* I-digested and alkali-denatured SV40/ϕX174 recombinant DNA (same DNA as in *A*) supplemented with 10^6 cpm of heat-denatured ϕX174 [^{32}P]DNA (3.8×10^7 cpm/μg); strip b, 1.3 μg of *Hga* I-digested and alkali-denatured control DNA (as in *A*), supplemented with 10^6 cpm of heat-denatured ϕX174 [^{32}P]DNA. After autoradiography for 48 hr at room temperature, strip b was rehybridized with heat-denatured SV40 [^{32}P]DNA (17,000 cpm/ml; 5×10^7 cpm/μg) and reautoradiographed for 20 hr at room temperature (strip c).

was established by the experiment described in Fig. 3. Supercoiled DNA, isolated from cells 50 hr after infection with stock B of Exp. 59, was digested with *Hga* I, denatured by alkali treatment (0.2 M NaOH, 30 min at room temperature), and neutralized. This DNA was then either mixed with a SV40 [^{32}P]DNA probe and hybridized with immobilized ϕX174 DNA or mixed with a ϕX174 [^{32}P]DNA probe and hybridized with immobilized SV40 DNA. As a control, a mixture containing 1% authentic ϕX174 RF1 DNA and 99% authentic SV40 DNA was digested with *Hga* I, alkali-denatured, mixed with [^{32}P]DNA, and hybridized as above. If the progeny of stock B contains genuine SV40/ϕX174 recombinant DNA molecules, we expect (*i*) that those recombinant DNA molecules containing one or more *Hga* I sites will be converted by alkali to a single-stranded state (supercoiled wild-type SV40 DNA, which possesses no *Hga* I sites will not be denatured to single strands by this procedure) and (*ii*) that the single strands containing linked ϕX174 and SV40 DNA sequences will hybridize with both ϕX174 [^{32}P]DNA and filter-bound SV40 DNA or with both SV40 [^{32}P]DNA and filter-bound ϕX174 DNA. The results shown in Fig. 3 clearly demonstrate the linkage. Immobilized ϕX174 DNA bound the SV40 [^{32}P]DNA probe when incubated together with the *Hga* I-digested alkali-denatured sample of the recombinant DNA population (Fig. 3*A*, strip a) but not when incubated together with the similarly treated control DNA (strip b). Strip b reacted strongly, as expected, when rehybridzed with ϕX174 [^{32}P]DNA (Fig. 3*A*, strip c). The reciprocal experiment is shown in Fig. 3*B*: In this case, the immobilized SV40 DNA bound the ϕX174 [^{32}P]DNA probe when incubated together with the *Hga* 1-digested alkali-denatured sample of recombinant DNA (duplicate strips a) but not when incubated with the similarly treated sample of the control DNA mixture (duplicate strips b). The fact that the [^{32}P]DNA probes, in both experiments, did not bond to heterologous immobilized DNA when incubated in the presence of the control DNA (authentic ϕX174 RF1 DNA unlinked to authentic SV40 DNA) excludes nonspecific trapping effects.

Cotransfection with SV40 DNA and other DNAs of diverse origin

Table 1 also lists the results of experiments in which BSC-1 cells were cotransfected with 5.2-kb SV40 DNA I and 5.2-kb polyoma virus DNA I or 4.3-kb plasmid pBR322 DNA I or 9.6-kb plasmid pMIA1 DNA I or linear pBR/A DNA (see footnote † in Table 1 for information on these DNAs). Representative autoradiograms of the putative recombinant plaques generated by the yields of those cotransfections are presented in Fig. 4. When SV40 DNA was omitted from the cotransfection mixture, the 10-day lysates of the transfected cultures yielded no foci that reacted with the appropriate [^{32}P]DNA probe in the infectious-center *in situ* plaque hybridization test.

The occurrence of putative recombinants between SV40 DNA and plasmid pMIA1 DNA is of note because this DNA is substantially larger (9.6 kb) than the other DNAs tested (4.3–5.2 kb). Because the [^{32}P]DNA probe in Exp. 53 contained sequences homologous to both pBR322 and mouse type A particle genes, we do not know which of these sequences is represented in the recombinant plaques. In the case of cotransfection with pBR/A DNA (Exp. 32), both pBR322 and mouse type A particle DNA sequences are represented in the recombinants, because cloning by plaque isolation generated two classes of populations; one class reacted only with the pBR/A [^{32}P]DNA probe, whereas the second class reacted with both the pBR/A and the pBR322 [^{32}P]DNA probes (data not shown).

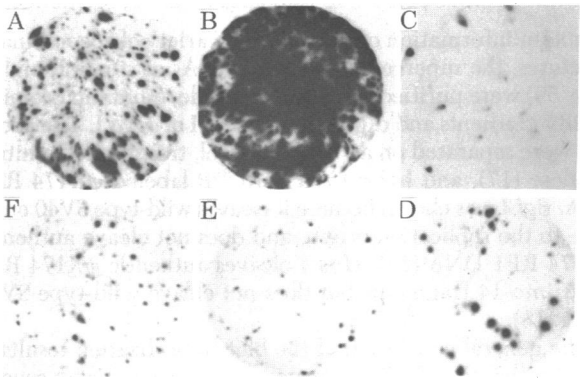

FIG. 4. Autoradiograms showing the plaques generated by different putative recombinant SV40 populations (see Table 1 for details). (*A* and *B*) SV40/polyoma virus recombinant plaques generated by virus harvested from cells cotransfected with SV40 DNA and polyoma virus DNA at, respectively, 1 or 5 μg/ml (Exp. 62); (*C*) SV40/pBR322 (Exp. 49); (*D*) SV40/pMIA1 (Exp. 53); (*E*) SV40/pBR/A stock A; and (*F*) SV40/pBR/A stock B (Exp. 32).

DISCUSSION

The cotransfection experiments with SV40 DNA and ϕX174 RF1 DNA have demonstrated that recombination between the DNAs of an animal and a bacterial virus can occur in mammalian cells. The restriction endonuclease cleavage patterns of the uncloned SV40/ϕX174 recombinant DNA populations indicate that a number of different recombinant structures arose. These will now have to be cloned and individually mapped to determine if particular regions of the ϕX174 genome are selected and which portions of the SV40 genome are retained. The cleavage pattern with *Bgl* I strongly suggests that, like the host-substituted SV40 variants (2–4, 19), many of the SV40/ϕX174 recombinants possess multiple origins for SV40 replication.

It is a common observation that transfection of monkey cells with SV40 DNA converts a maximum of only a few percent of the cells to virus production. Progeny virions arising from SV40 DNA tranfection then initiate multiple cycles of infection until all the cells have succumbed. The recombinant DNA structures, if they are to survive, must be of a size commensurate with encapsidation. Survival will also depend, presumably, on a sufficiency of wild-type SV40 to provide helper functions. It may be argued that the initial recombination event between SV40 DNA and ϕX174 RF1 DNA need not necessarily have occurred in the small subpopulation of cells successfully transfected at the beginning of the experiment. ϕX174 RF1 DNA (5386 nucleotide pairs) is sufficiently similar in size to SV40 DNA (5243 nucleotides) for it to be encapsidated, unlinked to SV40 DNA, into pseudovirion particles and in this form transmitted to other cells, where recombination may occur. Evidence against this possibility has been obtained from experiments in which the cell population was dispersed 5 hr after exposure to SV40 DNA and ϕX174 RF1 DNA and analyzed by infectious-center *in situ* plaque hybridization, using SV40 and ϕX174 [^{32}P]DNA probes. One-half percent of the cells registered as infectious centers with the SV40 DNA probe and 0.005% of the cells registered as infectious centers with the ϕX174 DNA probe. These measurements indicate that recombination does in fact occur in the initially transfected subpopulation, albeit in only 1% of the cells replicating SV40 DNA.

Although the linkage between SV40 DNA and polyoma virus DNA or bacterial plasmid DNA or the cloned segment of mouse genomic DNA (experiments 62, 49, 53, and 32 in Table 1) has not as yet been established by experiments of the type described for SV40/ϕX174 recombinants, several arguments show that the cotransfected DNA replicated in monkey cells and therefore, by inference, recombined with SV40 DNA. As calculated from the specific activity of the [^{32}P]DNA probes, a minimum of 1000 copies of the polyoma virus (wild-type polyoma virus DNA does not replicate in monkey cells) or plasmid or mouse DNA sequences must be present per monkey cell before a detectable reaction is obtained by hybridization and autoradiography. In addition, the size of the recombinant plaques increased with time of transfer to the nitrocellulose membrane filters (similar to the development of the SV40/ϕX174 recombinant plaques shown in Fig. 1 *A* and *B*) and no foci were detected when SV40 DNA was omitted from the cotransfection mixture. In the case of the SV40/pBR/A products, the number of putative recombinants increased upon serial passage (Table 1, Exp. 32). The only feasible explanation to account for the replication of the cotransfected DNA is to assume recombination with SV40 DNA. Conceivably, all that is required is linkage with an SV40 replication origin, the presence of helper virus, and the formation of a DNA molecule of a size commensurate with encapsidation. In this respect, the SV40/polyoma virus DNA products are of interest because growth of the putative recombinant virus in mouse cells, in the presence of wild-type polyoma helper virus, should select for recombinants that have retained a polyoma virus replication origin in addition to a SV40 origin.

The molecular nature of the recombination event that occurs in the cotransfected cells is, perhaps, the most challenging aspect that has to be investigated. It is hoped that the systems and techniques described in this report will contribute to an understanding of the process. Whatever the recombination mechanism, it is clearly unable to distinguish between DNAs of prokaryotic and eukaryotic origin.

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