JOURNAL OF VIROLOGY, Apr. 1972, p. 717-719

Vol. 9, No. 4
Printed in U.S.A.

Isolation of Simian Virus 40 Recombinants from Cells Infected with Oligomeric Forms of Simian Virus 40 Deoxyribonucleic Acid

DEL ROSE DUBBS, SAUL KIT, RUDOLF JAENISCH, AND ARNOLD J. LEVINE

Division of Biochemical Virology, Baylor College of Medicine, Houston, Texas 77025, and Department of Biochemistry, Princeton University, Princeton, New Jersey 08540

Received for publication 14 January 1972

Oligomeric forms of simian virus 40 (SV40) deoxyribonucleic acid (DNA) were isolated from monkey kidney cells infected with two plaque morphology mutants of SV40. Recombinant, large clear-plaque-type SV40 was produced in cells productively infected with oligomeric forms of SV40 DNA.

Repeated centrifugation through alkaline sucrose gradients has been employed to isolate monomers, dimers, and complex forms of simian virus 40 (SV40) deoxyribonucleic acid (DNA) (5). The dimers and higher oligomers of SV40 DNA might be generated by either an aberrant DNA replication process (2, 10) or as intermediates in recombination between circular molecules (4, 8). To distinguish between these two possibilities, the following experiment was performed. Secondary cultures of African green monkey kidney cells were mixedly infected with two plaque morphology mutants of SV40. The newly synthesized SV40 DNA was extracted by the

TABLE 1. Plaque morphology of progeny virus after infection of CV-1 cells with DNA fractions isolated from CV-1 cultures infected with one or two plaque morphology mutants of SV40[a]

DNA fraction	Description	Titers of SV40 DNA (PFU/ml)	No. of plaques tested	Progeny virus of picked plaques:			
				% Fuzzy	% Small clear	% Large clear	% Mixed[b]
A-I	Monomers[c]	1.1×10^7	70	34	26	0	40
A-II	Dimers[c]	1.6×10^4	57	42	32	0	26
A-III	Higher oligomers[c]	8.4×10^3	71	45	25	11[d]	18[d]
B-I	Monomers sc SV40 DNA[e]	8.8×10^6	25	0	100	0	0
C-I	Monomers f SV40 DNA[e]	3.0×10^6	23	100	0	0	0
B-I + C-I	Monomers f SV40 DNA + monomers sc SV40 DNA[e]		80	65	14	0	21
B-II + C-II	Dimers f SV40 DNA + dimers sc SV40 DNA[e]	1.4×10^4	69	55	28	0	17

[a] CV-1 = African green monkey kidney; PFU = plaque-forming units; f = fuzzy; sc = small clear.
[b] Mixed plaques are defined as plaques from which both fuzzy- and small clear-plaque-type viruses were isolated. With one exception[d] no large clear-plaque-type virus was isolated from mixed plaques.
[c] Isolated from mixed infection.
[d] The progeny of one of the 71 picked plaques was a mixed population of fuzzy- and large clear-plaque types. The other picked plaques which yielded large clear virus did not contain either fuzzy- or small clear-plaque virus.
[e] Isolated from single infection.

method of Hirt (3). Monomers (A-I), dimers (A-II), and a higher oligomer fraction (trimers-hexamers) (A-III) of SV40 DNA were isolated by repeated centrifugation in alkaline sucrose gradients (5). The various fractions were then assayed for infectivity on CV-1 (an established line of African green monkey kidney) monolayers (7). Plaques were picked at terminal dilutions and analyzed to determine the plaque morphology of the progeny virus. Let us assume that the A-II and A-III of SV40 DNA arise by recombinational events. Then, in the plaque assays with A-II and A-III fractions of SV40 DNA, the percentage of plaques yielding both parental types (heterozygotes), stable recombinants, or both, might be greater than in the plaque assays with mixture of A-I DNA species. For control purposes, cells were infected with each plaque morphology mutant alone, and monomer and dimer SV40 DNA fractions were isolated. DNA monomer fractions from each mutant (B-I and C-I) were then mixed in vitro and analyzed in a similar manner. DNA dimer fractions (B-II and C-II) from each mutant were also mixed in vitro and studied.

The two plaque morphology mutants of SV40 used were SV40(mKS-U4) which produces fuzzy plaques and SV40(3T3-4-88J3) which produces small clear plaques on CV-1 cells (reference 1; Fig. 1A, 1B). DNA samples were dialyzed against phosphate-buffered saline lacking magnesium and calcium and were assayed on CV-1 monolayers with 500 μg of diethylaminoethyl cellulose (DEAE)-dextran/ml as previously described (7). Overlay medium for the plaque assay was McCoy modified "R5a" medium with 7.5% fetal calf serum and 1% Difco agar (1). The morphology of the plaques was noted. Then plaques were picked with capillary pipettes from plates at terminal dilutions, which usually contained one to five plaques, and were resuspended in 1.5 ml of the medium. The samples were treated by sonic oscillation at 10 kc at 4 C for 3 min. Serial dilutions were prepared and replated on CV-1 monolayers, and the progeny virus was scored as to morphology.

The results (Table 1) demonstrate that both small clear- and fuzzy-plaque mutants were obtained from 17 to 40% of the individual plaques produced by the A-I and A-II fractions isolated from mixed infection as well as with monomers or dimers mixed in vitro (B-I + C-I and B-II + C-II). In these instances, cells probably were infected with more than one DNA molecule. DEAE-dextran was used to enhance the infectivity of the SV40 DNA. One plaque-forming unit of SV40 DNA is equivalent to 10^4 to 10^6 SV40 DNA molecules (9). Since there are about 10^6 cells/plate, one would not expect to observe such a high incidence of mixed plaques unless the DEAE-dextran carried many DNA molecules into individual cells. Because of the high incidence of mixed plaques after infection with the A-I fraction or from mixed monomers (B-I + C-I) or mixed dimers (B-II + C-II), a definitive

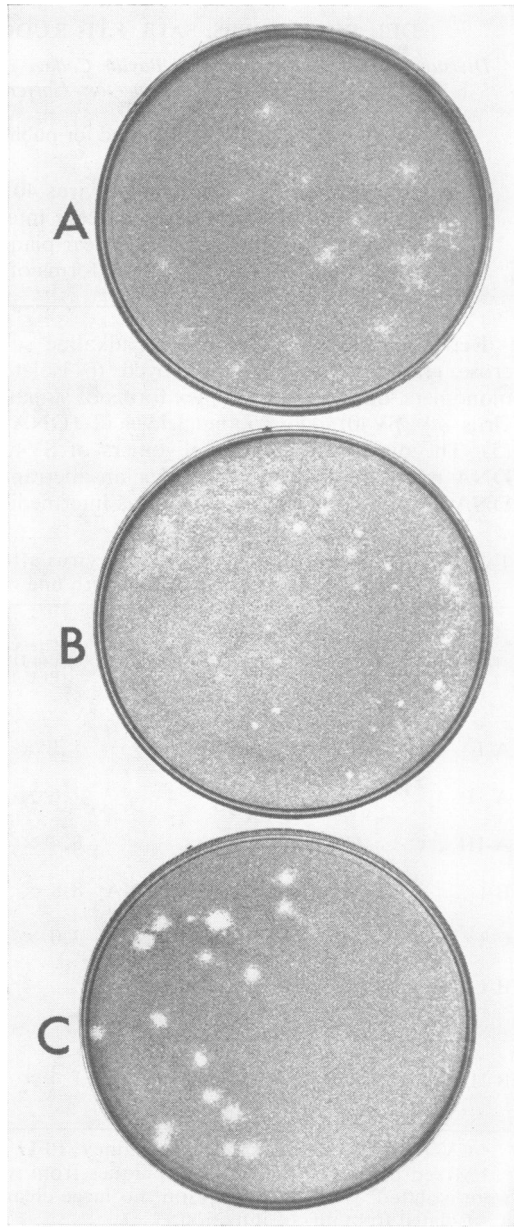

FIG. 1. *Plaque types. A, Fuzzy-plaque-type SV40 (mKS-U4); B, small clear-plaque-type SV40(4-88J3); C, large, clear-plaque-type recombinant SV40 isolated from higher oligomer fraction, A-III.*

answer to the question of how SV40 dimers and higher oligomers arise could not be achieved.

An unexpected result was obtained when CV-1 cells were infected with the A-III of SV40 DNA isolated after mixed infection. In this case, 45% of the plaques yielded only the fuzzy-type virus, 25% yielded only the small clear-plaque type, 18% yielded both fuzzy- and small clear-plaque types, but 11% of the plaques produced by the A-III fraction were large and clear (Fig. 1C). When these large clear plaques were picked and the progeny virus was replated, the progeny virus continued to produce large clear plaques, indicating that the change was stable. No large clear-plaque virus was isolated from plaques produced by DNA fractions A-I or A-II, although these fractions were isolated from the same mixedly infected cultures as A-III. Moreover, no large clear-plaque-type virus was isolated from monomer fractions obtained from single infection (B-I or C-I) or when B-I and C-I or B-II and C-II fractions (dimers from single infections) were mixed in vitro prior to plating on CV-1 cells. These results suggest that the large clear-plaque-type was not simply a revertant of the fuzzy- or small clear-plaque-type virus, but rather a stable recombinant.

The mechanism by which the recombinants were derived from the complex SV40 oligomers is not known. Either the A-III SV40 DNA forms were heterozygotes (containing both plaque morphology mutants in the same molecule) or recombination occurred in those cells jointly infected with the A-III of each parental type. In the former case, a recombinant DNA could have been generated during the conversion of A-III to a monomer (6). In the latter case, oligomeric forms of SV40 DNA may have replicated in the cells (6) and recombinants were then generated from the progeny DNA multimeric or monomeric circles. Experiments are now in progress to characterize the DNA and capsid proteins of the recombinant virus.

LITERATURE CITED

1. Dubbs, D. R., and S. Kit. 1970. Isolation of double lysogens from 3T3 cells transformed by plaque morphology mutans of SV40. Proc. Nat. Acad. Sci. U.S.A. 65:536–543.
2. Gilbert, W., and D. Dressler. 1968. DNA replication: the rolling circle. Cold Spring Harbor Symp. Quant. Biol. 33:473–484.
3. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. 26:365–369.
4. Hudson, B., and J. Vinograd. 1967. Catenated circular DNA molecules in HeLa cell mitochondria. Nature (London) 216:647–652.
5. Jaenisch, R., and A. Levine. 1971. DNA replication in SV40-infected cells. V. Circular and catenated oligomers of SV40 DNA. Virology 44:480–493.
6. Jaenisch, R., and A. J. Levine. 1971. Infection of primary African green monkey cells with SV40 monomeric and dimeric DNA. J. Mol. Biol. 61:735–738.
7. Kit, S., T. Kurimura, M. L. Salvi, and D. R. Dubbs. 1968. Activation of infectious SV40 DNA synthesis in transformed cells. Proc. Nat. Acad. Sci. U.S.A. 60:1239–1246.
8. Rush, M. G., and R. C. Warner. 1968. Multiple length rings of φX 174 and S13 replicative forms. III. A possible intermediate in recombination. J. Biol. Chem. 243:4821–4828.
9. Trkula, D., S. Kit, T. Kurimura, and K. Nakajima. 1971. Infectivity of molecular forms of simian virus 40 DNA. J. Gen. Virol. 10:221–229.
10. Yoshikawa, H. 1967. Initiation of DNA replication in *Bacillus subtilis*. Proc. Nat. Acad. Sci. U.S.A. 58:312–319.