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Emergence of Simian Virus 40 Variants During Serial Passage of Plaque Isolates

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Three serial passage series of simian virus 40 (SV40) in CV-1 cells were initiated by infection directly from the same wild-type plaque isolate, three series were initiated by infection with another plaque isolate, and two series were initiated with each of two other plaque isolates. Aberrant SV40 genomes were not detected in any of the passage series until after the fifth undiluted passage, and each series generated a different array of variant genomes. The results show that the variants were not present in the original plaque isolates but, instead, were randomly generated during subsequent high-input multiplicity passages. Although many of the aberrant viral genomes in each passage series contained reiterations of the SV40 origin of replication and some also contained host cell sequences, there was no indication that SV40 is predisposed toward generating any particular variant.

We recently reported that undiluted serial propagation of simian virus 40 (SV40) in the CV-1 line of African green monkey kidney cells results in detectable levels of aberrant viral genomes by only the second undiluted passage (13). Those passage series were initiated by infection at 100 PFU per cell with plaque-purified wild-type (WT) virus prepared at low multiplicity of infection (MOI). We also noted that seed amounts of aberrant genomes in SV40 stocks are amplified to detectable levels during only one further undiluted passage in CV-1 cells. Thus, we wanted to determine when aberrant SV40 genomes would arise during undiluted serial propagation of plaque isolates. Also, there is evidence that SV40 might be predisposed toward generating a particular defective genome comprised of reiterations of a sequence encompassing the SV40 replication origin and particular monkey cell sequences (9, 15, 16). Thus, we also looked for the generation of aberrant viral genomes common to independent passage series.

Eight undiluted serial passage series of SV40 (strain 777) in CV-1 cells were established as follows. Three series (1A, 1B, and 1C) were initiated by infection directly from the same plaque isolate, three series (2A, 2B, and 2C) were initiated by direct infection from another plaque isolate, and two series (3 and 4) were initiated from each of two other plaque isolates. The initial plaque isolates were picked into 0.5 ml of medium and were used to inoculate CV-1 monolayer cultures (0.05 ml of inoculum per 35-mm culture dish). Lysates from passage 1 and all following passages were harvested at 72 h, sonicated, and used undiluted as the inoculum for subsequent passages (0.05 ml of inoculum per 35-mm culture dish).

Aberrant viral genomes first became apparent in the viral DNA samples between passages 5 and 10, as indicated by polyacrylamide gel electrophoresis of *Hin*dIII digests of the samples (Figs. 1A and B). At least one passage series, 2A, generated aberrant genomes by passage 8 (Fig. 1B, lane 12). Each passage series generated a different array of aberrant *Hin*dIII restriction fragments (Figs. 1B and C), with the number and intensity of the aberrant bands increasing with subsequent passages.

Because of the results reported here and because of the previously noted capacity of CV-1 cells to rapidly amplify seed amounts of aberrant SV40 genomes to detectable levels (13), we conclude that aberrant SV40 genomes did not arise in the passage series described here until passage 5 or thereafter. Furthermore, these variants arose in a random manner, and there was no indication that SV40 tended to generate a particular defective. It is clear that aberrant virions were not present in the initial plaque isolates, since, as noted by Holland et al. (5), an excellent test for determining whether a viral stock is free of aberrant genomes is to determine whether independent serial passage series from that stock generate the same or different sets of aberrant genomes.

As noted above, we previously reported that SV40 generates detectable levels of aberrant genomes by only passage 2 in series initiated at

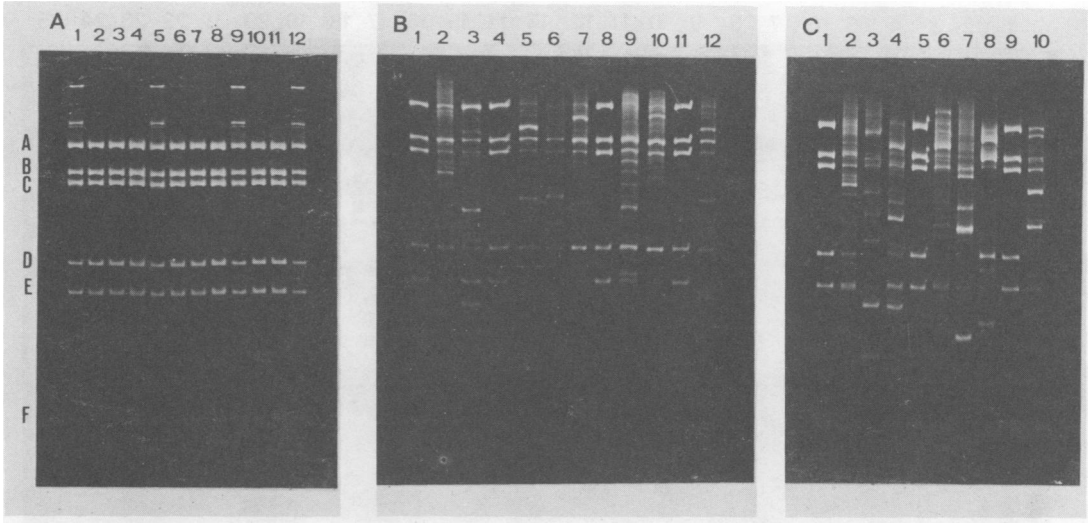

FIG. 1. Polyacrylamide gel electrophoresis of *Hin*dIII-restricted SV40 DNA samples from serial passages in CV-1 cells. (A) Lanes 1, 5, 9, and 12 contain WT SV40 DNA. Lanes 2, 3, 4, 6, 7, 8, 10, and 11 contain passage 5 samples of series 1A, 1B, 1C, 2A, 2B, 2C, 3, and 4, respectively. (B) Lanes 1, 4, 8, and 11 contain WT SV40 DNA. Lanes 2, 3, 5, 6, 7, 9, and 10 contain passage 10 samples of series 1A, 1C, 2A, 2B, 2C, 3, and 4, respectively. Lane 12 contains viral DNA from series 2A harvested after passage 8. (C) Lanes 1, 5, and 9 contain WT SV40 DNA. Lanes 2, 3, 4, 6, 7, 8, and 10 contain passage 15 samples of series 1A, 1B, 1C, 2A, 2B, 2C, and 4, respectively. Viral DNA preparation and gel electrophoresis were performed as previously described (12).

high MOI. In the present study, passages 1, 2, and 3 were at relatively low MOI, whereas passages 4 and 5 were at high MOI. For example, passages 2 to 5 of series 2A were at 1.4, 7, 180, and 430 PFU per cell, respectively. Thus, the results reported here and those of the earlier study suggest that high-input-multiplicity infections are necessary for SV40 to readily generate aberrant virions. Furthermore, we caution that SV40 stocks prepared from high-input-multiplicity infections are likely to contain aberrant genomes.

The viral DNA sample from passage 10 of series 2A appeared to contain an excess of genomes with an aberrant or missing *Hin*dIII fragment E (Fig. 1B, lane 5). Several small plaque isolates from this sample were restricted with *Hin*dIII, and all of these generated only standard-length *Hin*dIII fragments (not shown). Thus, those genomes which generated *Hin*dIII digests with an aberrant or missing fragment E are probably defective. Fragment E contains the overlapping sequences which encode the minor virion proteins VP2 and VP3 (4, 17).

Viral DNA samples from passage 15 of each series were further characterized by electrophoretic analysis in agarose gels (Fig. 2). The most rapidly migrating band of the unrestricted WT sample corresponds to supercoiled (form I) DNA and the other to relaxed circular (form II) DNA (18). Whereas the unrestricted WT sample displayed homogeneous bands, each of the unrestricted serial passage samples was heterogeneous. Restriction of the WT sample with the "single-cut" endonucleases *Eco*RI or *Bgl*I converted the circular WT genomes to homogeneous full-length linear (form III) molecules. In contrast, *Bgl*I restriction of the serial passage samples produced relatively large amounts of short fragments which were characteristic of each passage series. Because *Bgl*I makes a single cut on the WT SV40 genome at 0.67 map units (B. S. Zain and R. J. Roberts, unpublished results) at the origin for DNA replication (1, 3), the above result indicates that many of the aberrant genomes in each passage series contain reiterations of the origin sequence. Thus, although we have not found SV40 predisposed toward generating a particular variant, we find, in agreement with others, that this virus does tend to generate a class of defectives which contain reiterations of sequences encompassing the viral replication origin (e.g., see reviews by Fareed and Davoli [2] and Kelly and Nathans [6]). This defective class probably becomes predominant because it is able to initiate replication more frequently than the standard virus (8). In contrast, many of the aberrant genomes appear to lack the *Eco*RI cleavage site at map coordinates 0.0/1.0, within the coding region for the major viral capsid protein VP1 (7, 10, 11). However, there are a few viral genomes with multiple *Eco*RI restriction sites (e.g., in series 1C; Fig. 2, lane 12).

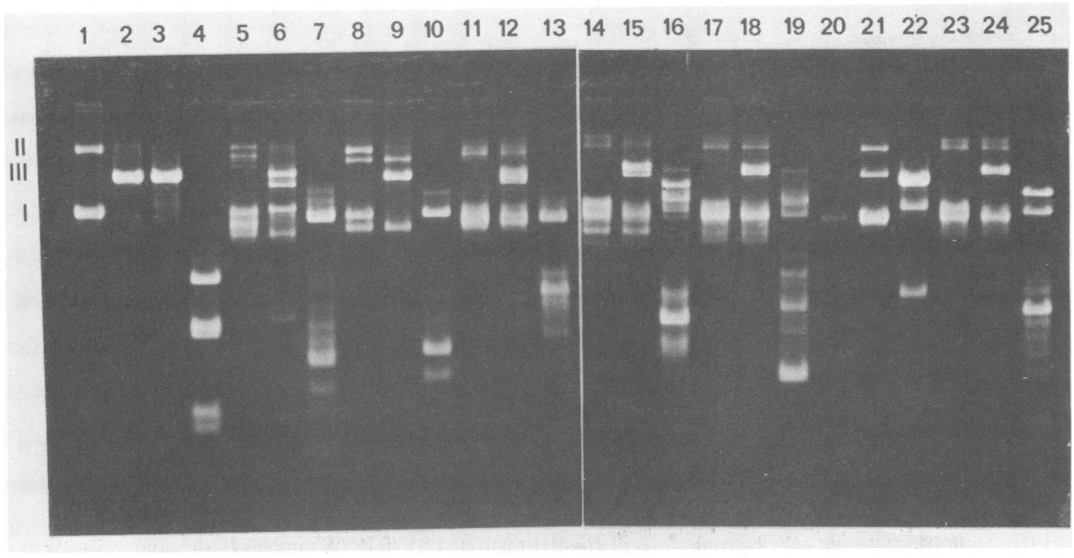

FIG. 2. Agarose gel electrophoresis of SV40 DNA samples from passage 15 in CV-1 cells. Lanes 1 through 4 contain WT SV40 DNA, lanes 5 through 7 contain a series 1A sample, lanes 8 through 10 contain a series 1B sample, lanes 11 through 13 contain a series 1C sample, lanes 14 through 16 contain a series 2A sample, lanes 17 through 19 contain a series 2B sample, lanes 20 through 22 contain a series 2C sample, and lanes 23 through 25 contain a series 4 sample. The samples in lanes 1, 5, 8, 11, 14, 17, 20, and 23 were unrestricted. The samples in lanes 2, 6, 9, 12, 15, 18, 21, and 24 were restricted with *Eco*RI. The samples in lanes 3, 7, 10, 13, 16, 19, 22, and 25 were restricted with *Bgl*I. The sample in lane 4 was restricted with *Hin*dIII. Viral DNA preparation and gel electrophoresis were performed as previously described (12).

High-input multiplicity serial passage of SV40 also tends to generate variants containing cellular DNA sequences. Consequently, viral DNA samples from passage 15 of each series were tested for the presence of host cell sequences by restriction with endonucleases *Sma*I and *Sst*I. These enzymes find no recognition sites within the WT SV40 genome and therefore cleave only those viral genomes which have incorporated cellular sequences or which have generated cleavage sites by mutation. The results (Fig. 3) show that viral genomes with *Sma*I restriction sites are present in passage series 2B and C (Fig. 3B, lanes 9 and 12), and perhaps in series 1A and 1C (Fig. 3A, lanes 6 and 12) as well. The series 2C sample also contains *Sst*I-sensitive viral genomes (Fig. 3B, lane 11). Viral DNA samples from the other passage series either do not contain *Sma*I or *Sst*I recognition sites or viral genomes with these sites are present in very low relative amounts. Other "no-cut" endonucleases might also reveal cellular DNA sequences in other of our passage series samples.

It might be expected that variants from independent passage series incorporate similar sequences which are highly reiterated in the monkey genome. However, there are reports that a particular infrequently reiterated monkey cell sequence also tends to be incorporated into SV40 genomes (9, 15, 16). These host cell sequences, and a portion of the SV40 genome which includes the origin, make up a sequence which is reiterated in several similar independently isolated defective SV40 variants. These findings are in contrast to the results reported here, in which we found no evidence for the independent generation of particular aberrant viral genomes. Earlier blot hybridization analysis of viral DNA samples from serial passage series and persistent infections generated in this laboratory failed to detect either the highly reiterated or infrequently reiterated monkey cell sequences which were incorporated into viral DNA during independent serial passage of SV40 in other laboratories (12, 13). These and other results (14) indicate that much remains to be learned about the variables which affect the generation of aberrant SV40 genomes.

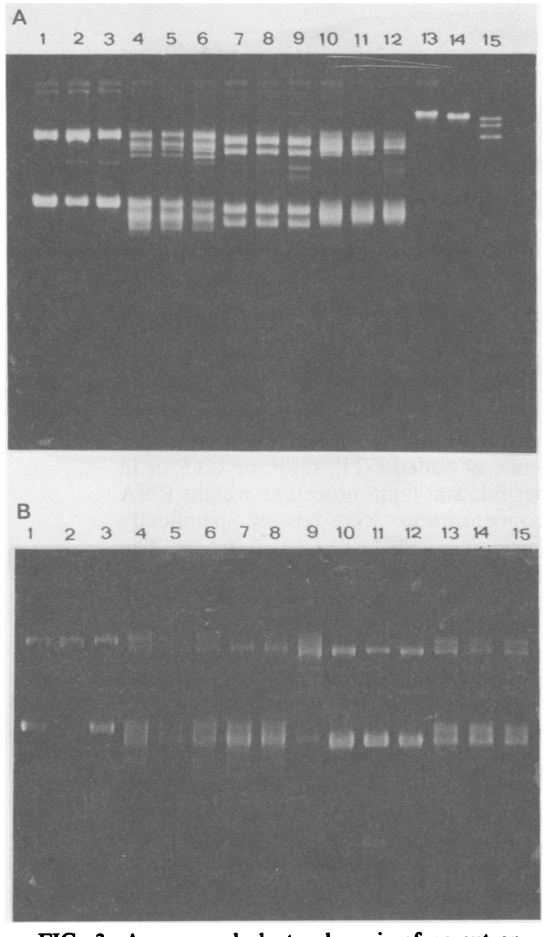

FIG. 3. Agarose gel electrophoresis of no-cut endonuclease-restricted SV40 DNA samples from passage 15 in CV-1 cells. (A) Lanes 1 through 3 contain WT SV40 DNA, lanes 4 through 6 contain a series 1A sample, lanes 7 through 9 contain a series 1B sample, lanes 10 through 12 contain a series 1C sample, and lanes 13 through 15 contain a λ-phage DNA sample. The samples in lanes 1, 4, 7, 10, and 13 are unrestricted. The samples in lanes 2, 5, 8, 11, and 14 were restricted with *Sst*I. The samples in lanes 3, 6, 9, 12, and 15 were restricted with *Sma*I. Note that *Sst*I cleaves λ-DNA at 51.7 and 53.9 map units on the λ-genome. *Sma*I cleaves the λ-genome at 40.7, 65.3, and 82.4 map units. (B) Lanes 1 through 3 contain WT SV40 DNA, lanes 4 through 6 contain a series 2A sample, lanes 7 through 9 contain a series 2B sample, lanes 10 through 12 contain a series 2C sample, and lanes 13 through 15 contain a series 4 sample. The samples in lanes 1, 4, 7, 10, and 13 are unrestricted. The samples in lanes 2, 5, 8, 11, and 14 were restricted with *Sst*I. The samples in lanes 3, 6, 9, 12, and 15 were restricted with *Sma*I. Viral DNA preparation and gel electrophoresis were performed as previously described (12).

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