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Isolation and Characterization of Defective Simian Virus 40 Genomes Which Complement for Infectivity

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A new variant of simian virus 40 (EL SV40), containing the complete viral DNA separated into two molecules, was isolated. One DNA species contains nearly all of the early (E) SV40 sequences, and the other DNA contains nearly all of the late (L) viral sequences. Each genome was encircled by reiterated viral origins and termini and migrated in agarose gels as covalently closed supercoiled circles. EL SV40 or its progenitor appears to have been generated in human A172 glioblastoma cells, as defective interfering genomes during acute lytic infections, but was selected during the establishment of persistently infected (PI) green monkey cells (TC-7). PI TC-7/SV40 cells contained EL SV40 as the predominant SV40 species. EL SV40 propagated efficiently and rapidly in BSC-1, another line of green monkey cells, where it also formed plaques. EL SV40 stocks generated in BSC-1 cells were shown to be free of wild-type SV40 by a number of criteria. E and L SV40 genomes were also cloned in the bacterial plasmid pBR322. When transfected into BSC-1 cell monolayers, only the combination of E and L genomes produced a lytic infection, followed by the synthesis of EL SV40. However, transfection with E SV40 DNA alone did produce T-antigen, although at reduced frequency.

Defective interfering particles (DIs) of numerous viruses arise during high-multiplicity passage in cultured cells (13). High multiplicity or undiluted passage has been thought to be required for the amplification of most DIs. An exception to this is simian virus 40 (SV40), where DIs arise during low-multiplicity or undiluted passage in a variety of human and monkey cell lines (17, 18). One such line is A172, derived from human glioblastoma cells (9). A172 cells yield DIs during low-multiplicity passage of SV40, and nearly all cells are destroyed. In addition, A172-derived DIs, unlike SV40 DIs generated in green monkey cells, contain reiterations of both the viral origin and the viral terminus (4, 5). However, when SV40 is passaged undiluted in A172, although DIs are still amplified, cell killing is abrogated, and carrier cultures or persistently infected (PI) cells emerge (17, 18). Virus lysates from acutely infected A172 cells or PI A172/SV40 cells are replete with DIs and can be used to establish PI cultures of TC-7 or BSC-1 green monkey cells. In the present report, we describe a novel class of SV40 DIs, derived from PI A172/SV40 and PI TC-7/SV40 cultures. These DI stocks were free of detectable wild-type nondefective virus and propagated most efficiently in BSC-1 cells. There are two types of DIs. One contains all or nearly all of the early (E) SV40 sequences, encircled by multiple viral origin and termination sequences. The second DI contains the late (L) SV40 sequences encircled by multiple origins and termini. Each DI was unable to productively infect BSC-1 cells alone but could do so when applied in combination.

MATERIALS AND METHODS

Cells and virus. TC-7 and BSC-1 are continuous lines of African green monkey kidney cells (12, 22). A172 is a human glioblastoma cell line (9). Cells were propagated in Eagle minimum essential medium with 5% bovine or fetal bovine serum.

The large-plaque strain of SV40 has been described previously (8, 17). The generation of triply plaque-purified SV40 has also been described (17, 18). Plaque purification of early-late (EL) SV40 was performed as described previously for wild-type SV40 (17).

Generation of PI cell lines. (i) A172 cells. Virus stocks were prepared after six and then again after nine diluted passages of SV40 in A172 cells (A172/SV40, passage 6; A172/SV40, passage 9; reference 17). A172/SV40, passage 6, was then passaged undiluted in A172 twice. The numerous survivors of this second undiluted infection grew continuously as virus-producing carrier cultures. They contained both wild-type and DI SV40 and were approximately 50% T-antigen positive (O'Neill and Carroll, submitted for publication).

(ii) TC-7 cells. A172/SV40, passage 9, and virus from PI A172/SV40 cultures were passaged undiluted in TC-7 cells twice. After the second passage, a small number of survivors propagated as PI cultures. These

cultures were designated PI TC-7/SV40-1 and PI TC-7/SV40-2. They contained both wild-type and DI SV40 and were, initially, approximately 50% T-antigen positive. However, after extensive subcultivation for periods of 8 to 12 months, they appeared to lose the wild-type viral genome, T-antigen, and infectious virus (O'Neill and Carroll, submitted for publication). PI TC-7/SV40-3 was established after infection with SV40 DIs generated during 16 serial undiluted passages of triply plaque-purified SV40 in TC-7 cells.

T-antigen analyses. SV40 intranuclear T-antigen was identified by indirect immunofluorescence, with serum from tumor-bearing hamsters, produced from SV40-transformed cells (19).

Assay for infectious virus. SV40 PI cultures were assayed for the presence of infectious virus by adsorbing dilutions of lysates onto BSC-1 cells. BSC-1 cells were utilized since viruses from PI TC-7/SV40 cultures often produced secondary PI cultures with little or no killing of TC-7 but produced massive cell destruction of BSC-1.

Extraction purification, and analysis of viral DNAs. Viral DNA was extracted and purified from infected cells by modifications (4, 17) of the methods of Hirt (11) and Boyd and Butel (1). Viral DNAs were then displayed on 1% agarose gels by electrophoresis (17). Preliminary identification of defective viral DNAs was made by the detection of faster-moving viral DNA bands on ethidium bromide-stained agarose gels (17, 18).

Restriction analyses of viral DNAs. *BglI*, *Bam*HI, *MspI*, *Eco*RI, and *TaqI* are endonucleases which produce single cleavages at unique sites of SV40 DNA. *BglI* cleaves SV40 DNA at the origin of replication (0.667 map unit), whereas *Bam*HI cleaves it at the terminus, the site opposite the viral origin (0.144 map unit). *MspI* produces a single cut at the late side of the origin (0.725 map unit). *Eco*RI and *TaqI* cleave within the late and early coding regions, respectively (1.0 and 0.566 map units). Defective viral DNAs with multiple origins or multiple termini yield one or more small (faster-moving) bands when cleaved with *BglI* or *Bam*HI. The small bands appear when there are multiple copies of the viral origin (*BglI*) or the viral terminus (*Bam*HI) arranged in tandem (4, 5). Larger fragments are also produced by *BglI* or *Bam*HI cleavage, and these contain sequences other than those in the origins and termini. These larger bands will be further cleaved by *Eco*RI or *TaqI* if they contain the L or the E SV40 coding sequence, respectively. *PstI* cleaves SV40 DNA at two sites, once in the early region and once in the late region.

Cloning of SV40 DIs. SV40 DNA from infected BSC-1 cells was isolated from agarose gels and ligated into the *Escherichia coli* plasmid pBR322 by modifications of previously described methods (10, 26). Plasmid and viral DNAs were cleaved with *PstI* and ligated together for transformation by T4 DNA ligase. Ligated DNA was used to transform *E. coli* HB101 (7). Clones with inserted SV40 sequences were identified by tetracycline resistance and ampicillin sensitivity. Inserted clones were further characterized by restriction analysis of plasmid DNA. Clones with E or L viral sequences were amplified in HB101, and plasmid DNA was extracted by a modified Hirt procedure (6). Inserted viral sequences were then removed by *PstI* cleavage and self-ligated at low DNA concentrations. These DNAs were used for the transfection of BSC-1 cells. For restriction analysis of viral inserts, plasmid DNAs were cleaved with *PstI* plus *ThaI* (*ThaI* cleaves pBR322 24 times and does not cleave the SV40 defectives). The viral sequences were separated from the plasmid sequences in 5 to 30% sucrose gradients.

Transfections with viral DNAs. SV40 viral DNAs were transfected into BSC-1 or TC-7 cells by a modification of the DEAE-dextran method (14), as previously described (17).

RESULTS

Analysis of SV40 defectives in PI A172 and TC-7 cultures. All PI A172 and TC-7 cultures contained defective viral DNAs (data not shown, but restriction analyses of Fig. 1 indicate that many DIs are present in PI A172 cells). In PI A172 cultures, the defectives were heterogeneous, and no two cultures contained similar mobility patterns of defectives. PI A172 cultures also contained significant amounts of wild-type viral DNA. In PI TC-7 cultures, the pattern of DIs tended to be more homogeneous (see Fig. 3). In PI TC-7/SV40-1 and -2, two defective bands predominated after six to ten in vitro subcultivations. Wild-type viral DNA became barely detectable or undetectable. In PI TC-7/SV40-3, a single defective band became dominant, and wild-type viral DNA was maintained at significant levels during extensive subcultivation.

Restriction endonuclease analysis of DNA from a single PI A172 culture showed again a plethora of defectives (Fig. 1). However, *BglI-Bam*HI cleavage revealed an unusual fragment of approximately 330 base pairs (bp) which is uniquely shared by all EL SV40 genomes. In addition, two larger fragments, approximating the size of the wild-type early and late regions, were also found. *BglI-MspI* cleavage revealed another unusual fragment of 660 bp and a large fragment approximating the combined size of the wild-type late region plus one 330-bp terminus fragment. This was also found in PI TC-7/SV40-1 and -2 and in EL SV40-infected BSC-1 cells (see Fig. 4). The small *BglI-Bam*HI 330-bp fragment is also consistent with previous observations that DIs generated in A172 cells contained both multiple origins and multiple termini (4, 5). This fragment contains *HpaI* (0.169 map unit) and *BclI* (0.189 map unit) cleavage sites found in the wild-type SV40 terminus (data not shown). Digestion of viral DNA from an early passage of acutely infected A172 cells did not reveal the characteristic fragments (Fig. 1), but the 330- and 660-bp fragments were found in later passages of acutely infected cells (data not shown). Virus from these late passages was used to establish PI A172 cells.

Analysis of defectives in PI TC-7/SV40-1 and -2 cultures showed similar fragments of 330 and

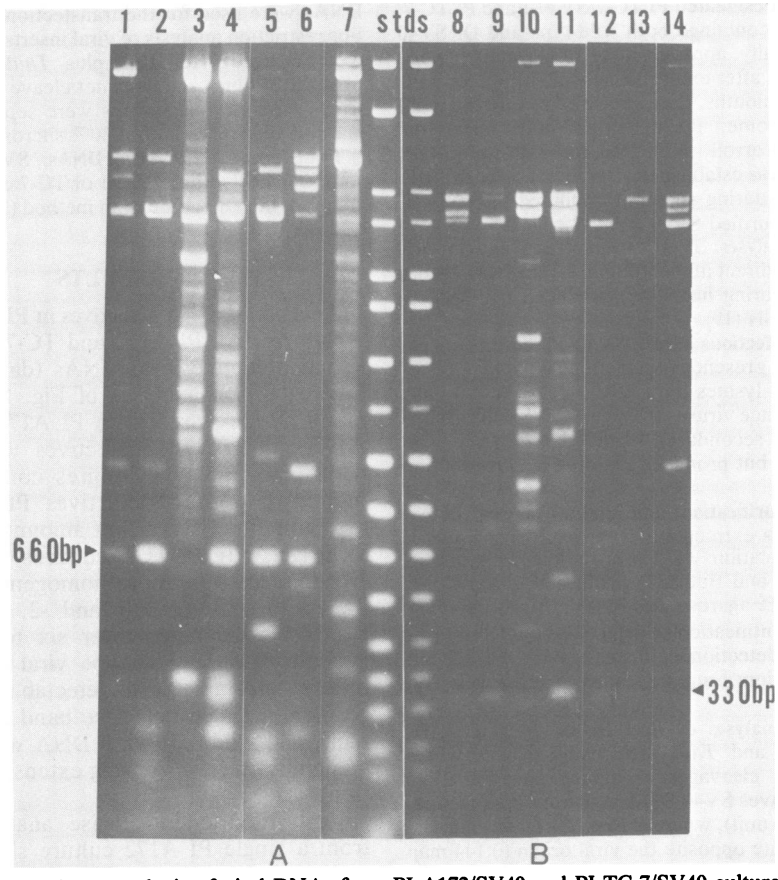

FIG. 1. Endonuclease analysis of viral DNAs from PI A172/SV40 and PI TC-7/SV40 cultures. (A) Lanes 1 and 2, *Bgl*I-*Msp*I cleavage of DNA from PI TC-7/SV40-1 and -2. Lane 3, *Bgl*I-*Msp*I cleavage of DNA from an early passage of an SV40 acute infection in A172 cells. Lane 4, *Bgl*I-*Msp*I cleavage of PI A172/SV40-1. Lanes 1, 2, and 4 reveal the characteristic 660-bp fragment. Lanes 5 and 6, *Bgl*I-*Msp*I cleavage of different passages of PI TC-7/SV40-1 and -2, revealing characteristic 660-bp fragments. Lane 7, *Bgl*I-*Msp*I cleavage of DNA from PI TC-7/SV40-3 (not derived from A172-grown SV40). SV40 size standards are shown between lanes 7 and 8. (B) Lanes 8 through 14, *Bgl*I-*Bam*HI cleavage of the same viral DNAs and in the same order as shown in (A). Lanes 8, 9, and 11 through 13 show characteristic 330-bp fragments for EL SV40 found in PI TC-7/SV40 cells generated in PI A172/SV40 cultures. Lane 10 (like lane 3) is DNA from A172 cells acutely infected at passage 6. Lane 14 (like lane 7) is PI TC-7/SV40-3, not derived from A172-grown SV40. Lanes 10 and 14 do not reveal the 330-bp fragment.

660 bp when cleaved with *Bgl*I-*Bam*HI and *Bgl*I-*Msp*I, respectively (Fig. 1). *Bgl*I-*Bam*HI cleaved all defectives in these PI cultures, yielding not only the 330-bp fragment but larger fragments as well. As shown by *Bgl*I-*Bam*HI and *Bam*HI-*Msp*I digestion, these larger fragments approximated the size of the entire E and the entire L viral sequence (Fig. 2A).

Further analysis of these defectives was made with the enzymes *Eco*RI and *Taq*I, each of which was used to cleave the defectives in combination with *Bgl*I (Fig. 2B). *Eco*RI cleaved two or perhaps three of the defectives, and *Taq*I cleaved the remaining defective. These cleavage patterns suggested that in PI TC-7/SV40-1 or -2, one defective contained early SV40 DNA and the other contained late SV40 DNA and that both types of defectives were flanked by multiple origins and termini. These defectives were named EL for early-late.

The defectives in PI TC-7/SV40-3 presented a different picture. Digestion with *Bgl*I-*Bam*HI and *Bgl*I-*Msp*I did not reveal the characteristic 330- and 660-bp fragments (Fig. 1). We analyzed the predominant defective at culture passage 25, although after more extensive subculturing a faster-moving defective became predominant. This defective contains only reiterated origins (Fig. 1 and 2B). It contains single *Msp*I, *Bam*HI, and *Eco*RI sites, but no *Taq*I site, suggesting that it contains at least some late SV40 sequences.

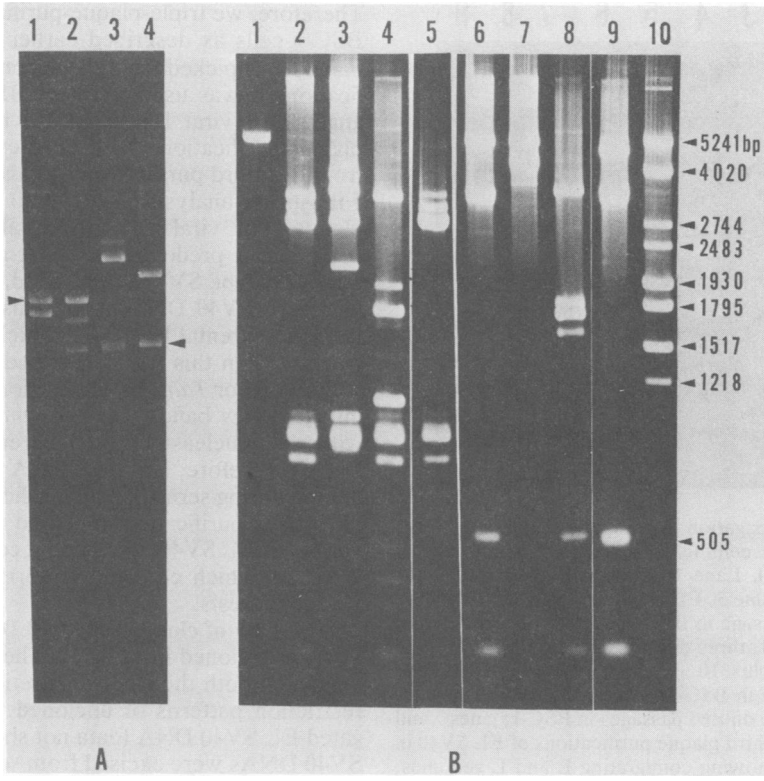

FIG. 2. Further restriction endonuclease analyses of EL SV40 from PI TC-7/SV40 and defective SV40 from PI TC-7/SV40-3. (A) Lane 1, *BglI-BamHI* cleavage of wild-type SV40; lane 2, EL SV40 DNA; lane 3, *BamHI-MspI* cleavage of EL SV40; and lane 4, wild-type SV40 DNA. Arrows indicate comigrating early (lanes 1 and 2) and late (lanes 3 and 4) fragments. The faster-moving bands of lane 3 are a doublet, only one of which comigrates with the fragment from wild-type DNA. (B) Lane 1, *EcoRI*, wild-type SV40; lanes 2 through 5, cleavages of PI TC-7/SV40-1 by *BglI*, *BamHI*, *BglI-EcoRI*, and *BglI-TaqI*, respectively. Lanes 6 through 9, cleavages of PI TC-7/SV40-3 by *BglI*, *BamHI*, *BglI-EcoRI*, and *BglI-TaqI*, respectively. Lane 10, Size standards from wild-type SV40. Lanes 4 and 5 show the existence of 2 to 3 defectives with *EcoRI* cleavage sites and a single defective with a *TaqI* site. Lanes 8 and 9 show defectives with an *EcoRI* site but no *TaqI* site. The uppermost band in lane 5 (approximately 3,500 bp) comigrates with bands in lanes 2 and 4. This does not represent incomplete *TaqI* cleavage; rather it is an additional defective (with an *EcoRI* site), which coincidentally comigrates with the defective containing a *TaqI* cleavage site.

Propagation of EL DIs in BSC-1. Lysates from PI TC-7/SV40-1 and -2 cultures (containing EL virus) were allowed to infect monolayers of BSC-1 cells, diluted or undiluted. This resulted in the rapid appearance of cytopathic effect, and Hirt supernatants from infected cells contained large amounts of viral DNA. We were able to passage EL virus diluted in BSC-1 cells, without loss of infectivity and with maintenance of the integrity of the defective viral DNA bands (Fig. 3).

EL SV40 DNA derived from infections of BSC-1 cells with virus from PI TC-7/SV40-1 and -2 was analyzed by cleavage with *PstI*, *BglI*, *BamHI*, *MspI*, *EcoRI*, and *TaqI*. These data were used to formulate the genome maps in Fig. 4. Some of the data appear in Fig. 5, and some are not shown, but they were similar to those in Fig. 1 and 2. The results have shown that: (i) there was no detectable wild-type viral DNA, even when gels were overloaded, and (ii) there were two main types of defective SV40 species, one with the entire E sequence and the other with nearly the entire L sequence. Again, each defective was encircled by multiple origins and termini. The E and L viral sequences identified in acutely infected BSC-1 cells were very similar to those seen in PI TC-7 cells. The only difference in EL viruses derived from the two cell types was in the number of origins and termini and perhaps the size of some of the sequences in the origins and termini. This type of heterogeneity has also been found in defectives rescued from latently infected cultures of PI TC-7/SV40-1 and -2 (unpublished data). Since no wild-type SV40 DNA was detectable and, as we show

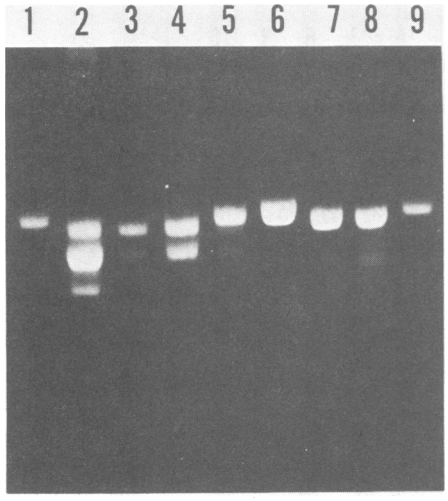

FIG. 3. Propagation and plaque formation of EL SV40 in BSC-1 cells in the presence and absence of wild-type SV40. Lane 1, wild-type SV40; lane 2, PI TC-7/SV40-1; lane 3, EL SV40, from PI TC-7/SV40-2, one diluted passage in BSC-1; lane 4, EL SV40, from PI TC-7/SV40-1, three diluted passages in BSC-1; lane 5, EL SV40 plus 10 PFU of wild-type SV40, one diluted passage in BSC-1; lane 6, EL SV40 plus wild-type SV40, two diluted passages in BSC-1; lanes 7 and 8, second and third plaque purifications of EL SV40 in BSC-1 cells, showing comigrating E and L genomes; lane 9, wild-type SV40.

below, it was absent, the EL DIs acted in concert to produce an infection.

Addition of triply plaque-purified SV40 to EL SV40 propagated in BSC-1 cells. When propagated in BSC-1 cells, EL SV40 contained no detectable wild-type viral DNA in Hirt supernatants. If wild-type SV40 is present at very low levels, the addition of very small amounts of triply plaque-purified SV40 should not alter the balance of EL SV40 with the low levels of putative wild-type SV40. EL SV40 was serially passaged in BSC-1 cells three times, at a dilution of 10^{-2}. In some experiments, at the time of the first passage, a total of 10 PFU of triply plaque-purified SV40 was added (10 PFU per culture). Viral DNA was extracted and analyzed after the first and second passages. As shown in Fig. 3, wild-type SV40 DNA was detected in significant amounts after a single passage of EL SV40 in the presence of 10 PFU of triply plaque-purified SV40. By the second passage, wild-type SV40 DNA predominated. After three passages of EL SV40 without added triply plaque-purified SV40, no wild-type SV40 DNA could be detected in Hirt supernatants.

Plaque purification of EL SV40. Serial plaque purifications have been shown to greatly reduce or eliminate DIs from virus stocks (17, 24). Therefore, we triple-plaque-purified EL SV40 in BSC-1 cells as described earlier (17). A single plaque was picked from the second plaque purification. It was used to infect BSC-1 cells, for analysis of viral DNA, and to initiate a third plaque purification. Two plaques were picked from this third purification and analyzed. Figure 5 illustrates analysis of EL SV40 from one such plaque, but viral DNA from all the plaques contained a predominant faster-moving (faster than wild-type SV40 DNA) band. There was no wild-type SV40 DNA band. This was different from the parental EL SV40, which contains two bands. When this plaque-purified band was cut with *Eco*RI or *Taq*I, only a portion of it was split into two new bands, each of which comigrated with endonuclease-cleaved parental EL SV40 DNA. Therefore, EL SV40 DNA was not lost or diluted during serial plaque purification. However, plaque purification appeared to select for a variant of EL SV40; this variant contained E and L DNAs which comigrated during agarose gel electrophoresis.

Infectivity of cloned EL SV40 DNA. EL SV40 DNA was cloned in pBR322. These viral DNAs contained both the electrophoretic mobility and restriction patterns of uncloned, BSC-1-propagated EL SV40 DNA (data not shown). E and L SV40 DNAs were excised from separate clones. They were then transfected into BSC-1 monolayers. Infection of BSC-1 with E or L cloned DNA did not produce an infection. But when BSC-1 cells were infected with both genomes, newly formed virus and cytopathic effect were produced, and EL SV40 DNA was present in Hirt supernatants (Table 1). Infection of BSC-1 with E DNA or with EL DNA resulted in the appearance of T-antigen. However, T-antigen was induced at greater frequency with wild-type SV40 DNA. L DNA did not induce T-antigen.

TABLE 1. Infectivity of E and L SV40 DNA cloned from pBR322 on BSC-1 cells[a]

DNA	CPE[b]	% Cells with T-antigen[c]	Viral DNA in Hirt supernatant[d]
Wild type	+	8–10	Wild-type only
E	−	3	—[e]
L	−	0	—
EL	+	3	E and L DNA only

[a] SV40 wild-type and EL DNAs were transfected into BSC-1 cells by the DEAE-dextran method.

[b] CPE, Cytopathic effect.

[c] For T-antigen analysis, monolayers of BSC-1 on cover glasses (22 by 22 mm) were transfected with 1.0 μg of viral DNA and harvested 48 h later.

[d] Viral DNA was prepared by infection of BSC-1 cells with virus lysates obtained 10 to 15 days after transfection by BSC-1 cells with indicated DNAs.

[e] —, None.

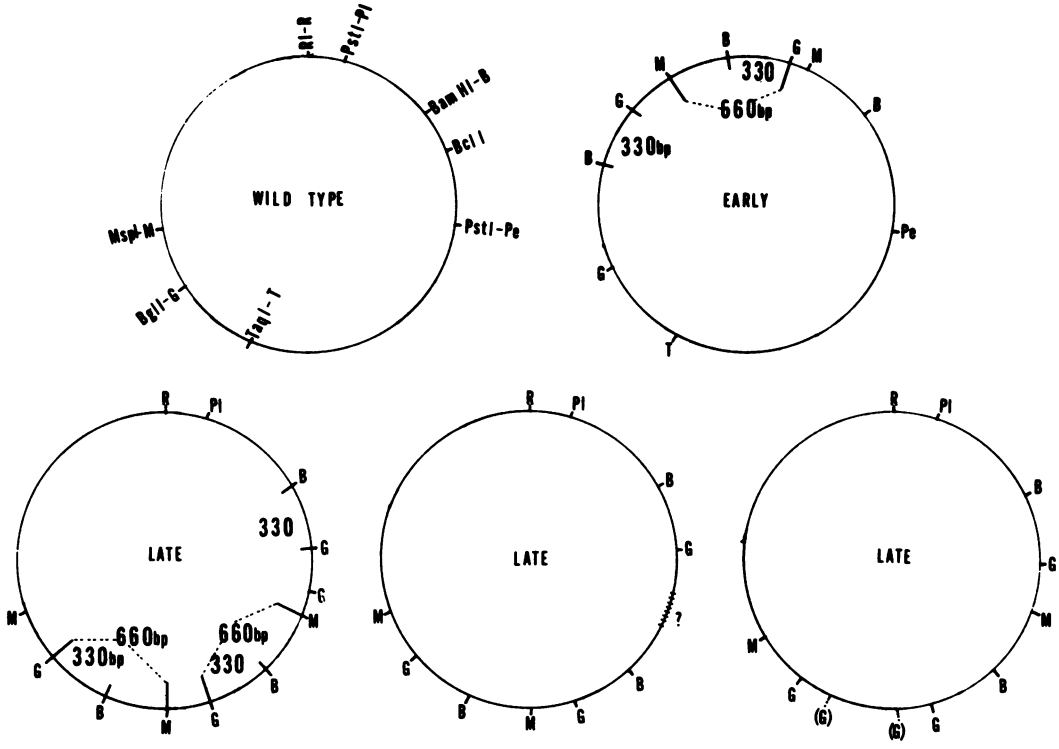

FIG. 4. Genome maps of a single E and three L SV40 genomes. Abbreviations: B (*Bam*HI), G (*Bgl*I), M (*Msp*I), R (*Eco*RI), T (*Taq*I), Pe (*Pst*I, early region site), and PI (*Pst*I, late region site).

DISCUSSION

A variant of SV40 was isolated which contained two separate but complementing genomes. One molecule contained all or nearly all of the E SV40 sequences, and the other contained the L viral sequences. Each genome was encircled by alternating viral origins and termini. Although EL SV40 was isolated from PI A172 SV40 and TC-7/SV40 cultures, it propagated most efficiently in BSC-1 cells. Infectivity of EL SV40 required the simultaneous presence of both E and L genomes. However, the E SV40 genome was capable of inducing T-antigen in transfected BSC-1 cells, although less efficiently than wild-type SV40 DNA.

A similar type of wild-type virus evolution may have occurred with the RF and MG variants of BK virus. RF and MG contain two species of viral DNA molecules (20, 21). In RF, one species contains at least 50% of the late region deleted, and the other contains at least 40% of the early region deleted from BK virus DNA (20). MG is more distantly related to prototype BKV but shows significant sequence homology (21). Sol et al. (23) have described variants of SV40 genomes arising during serial undiluted passage in BSC-1 cells. These variants contain early SV40 DNA and reiterations of the viral origin and probably the viral terminus. No variants containing late sequences were reported, and the early region variants were impaired in early lytic functions. The early region DIs produced T-antigen and caused transformation of nonpermissive cells. Evolutionary variants ev-1114, ev-1117, and ev-1119, described by Brockman et al. (2) and Woodworth-Gutai (28), resemble somewhat the SV40 genomes described here, in that they apparently contain either all of the early sequences (ev-1114) or all of the late sequences (ev-1117 and 1119).

In plants, numerous viruses have been identified which contain their genomes in two or three separate RNA molecules. These multipartite viruses are only infectious when permissive cells are infected with all of the different virions or virion RNAs (27).

Since the EL SV40 genomes are by themselves DIs, the question arose whether small amounts of wild-type virus are present in EL SV40 stocks. This putative but undetectable wild-type SV40 might be responsible for plaque formation and infectivity in BSC-1 cells. However, the results strongly suggest that EL SV40 stocks isolated in BSC-1 cells are free of wild-type SV40. (i) Wild-type SV40 DNA was unde-

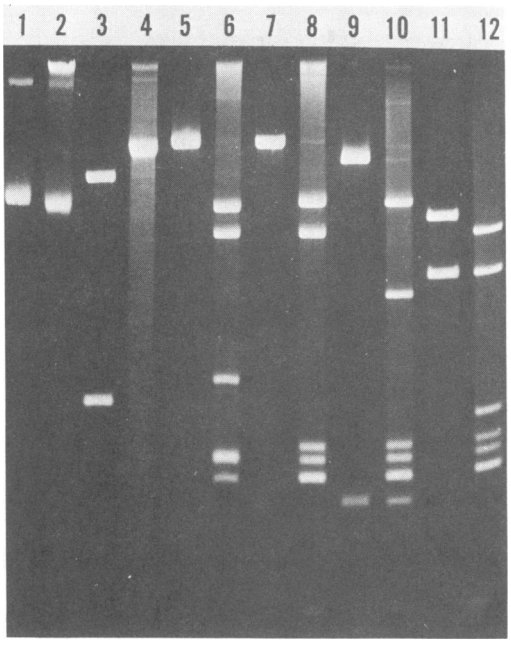

FIG. 5. Restriction analysis of plaque-purified EL SV40 genomes. Lane 1, Undigested wild-type SV40; lane 2, undigested prototype EL SV40; lane 3, *Pst*I, wild-type SV40; lane 4, *Pst*I, plaque-purified EL SV40; lane 5, *Bgl*I, wild type; lane 6, *Bgl*I, plaque-purified EL SV40; lane 7, *Bam*HI, wild type; lane 8, *Bam*HI, plaque-purified EL SV40; lane 9, *Bam*HI-*Eco*RI, wild type; lane 10, *Bam*HI-*Eco*RI plaque-purified EL SV40; lane 11, *Bam*HI-*Taq*I, wild type; lane 12, *Bam*HI-*Taq*I, plaque-purified EL SV40. Faint bands approximating the size of linear wild-type SV40 are seen in lanes 6 and 8. These represent partial endonuclease cleavage products. Their mobilities are retarded somewhat because of the presence of significant amounts of high-molecular-weight cellular DNA.

tectable in Hirt supernatants of EL SV40-infected BSC-1 cells, even when agarose gels were overloaded with viral DNA. (ii) Addition of very small amounts of wild-type SV40 (10 PFU per culture) to EL SV40 passaged in BSC-1 cells resulted in the diminution of EL SV40 and the emergence and dominance of wild-type SV40 DNA, within two passages. If small, undetectable amounts of wild-type SV40 are present in EL SV40 stocks, we argue that these would also overtake the defective genomes. (iii) Serial plaque purification, a method used to greatly reduce or eliminate DIs, also did not reveal the presence of wild-type SV40. However, plaque purification appeared to select for a variant of EL SV40, where the E and L genomes comigrated in agarose gel electrophoresis. (iv) Finally, we cloned EL SV40 DNA in the *E. coli* plasmid pBR322. Viral DNA excised from plasmid DNA was infectious in BSC-1 cells but only when cells are transfected with both E and L genomes.

We also performed similar transfection experiments with uncloned parental, BSC-1-propagated EL SV40 DNA. EL SV40 DNA was cleaved with *Eco*RI or *Taq*I. The cleaved DNAs were then separately and mixedly (*Eco*RI-*Taq*I cleaved) transfected into BSC-1 monolayers. Only the mixedly transfected DNAs produced plaques (data not shown).

EL SV40 appears to have originated in infected A172 cells. When A172-derived stocks were used to establish PI TC-7/SV40 cultures, the EL SV40 DIs were apparently preferentially selected. Although PI A172/SV40 cultures contained a variety of defective viral genomes, restriction endonuclease analysis revealed the presence of characteristic fragments, both large and small, found after cleavage of EL SV40 derived from TC-7 or BSC-1 cells. EL SV40 was not present in PI TC-7/SV40 cultures established from DI-containing SV40 stocks generated by undiluted passage in TC-7 cells (Fig. 1 and 2, and unpublished data). Therefore, EL SV40 was apparently generated in the cell type which uniquely produces DIs which contain, in addition to reiterated viral origins, reiterated viral termini (5).

What is the function of these reiterated sequences in EL SV40? The reiterated origins allow for effective competition of DIs with wild-type SV40, since they are shorter and contain more than one site for initiation of replication (3). As suggested previously, reiterated viral termini may function as terminators of early transcription (5). The replication advantage afforded to DIs by multiple origins might be counteracted by a disadvantage for transcription. Transcription initiated at an origin remote from the coding sequence might produce a faulty message, one which might interfere with normal transcription of DIs. However, multiple termini, adjacent to origins would prevent (terminate) transcription initiated at remote origins and ensure the competitive advantage for the DIs. Interestingly, both E and L genomes contain origins closest to the coding sequence, not interrupted by a termination sequence.

Certain questions remain unresolved. EL SV40 stocks would be expected to contain two types of virions. One type of virion would contain an E genome, and the other would contain an L genome. It is unlikely that both genomes could be packaged into a single normal virion since they contain 1.5 to 1.8 times the amount of wild-type SV40 DNA. If the EL genomes are separately packaged, they should exhibit "two-hit" kinetics for plaque formation in BSC-1 cells. We found that EL SV40 consistently exhibits "one-hit" kinetics, even when stocks have been extensively sonicated. Since EL SV40 exhibits interference activity, two-hit kinetics might be masked by simultaneous inter-

ference between E and L genomes. We have shown previously that DIs distort the normal kinetics of plaque formation by wild-type SV40 (17). The tripartite RNA plant viruses also exhibit one-hit kinetics for infectivity, although all cells must receive all of the different genomes for a successful infection to ensue (27). This problem and the structure of EL SV40 virions are currently being investigated.

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