

Multiple Insertions and Tandem Repeats of Origin-Minus Simian Virus 40 DNA in Transformed Rat and Mouse Cells

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Stable simian virus 40 (SV40) transformation requires integration and expression of the early region of the SV40 genome. We have examined the amount and state of integrated viral DNA of SV40-transformed NIH 3T3 mouse and F2408 rat fibroblast lines generated by transfection with either wild-type or origin-defective SV40 DNA. A functional SV40 replication origin was not required for multiple inserts and partial-repeat structures to form in NIH 3T3 mouse transformants. In contrast, partial repeats in F2408 rat transformants were rare when the SV40 replication origin was intact and not detected at all when it was defective.

When simian virus 40 (SV40) transforms a cell, at least part of its genome becomes integrated into the host cell DNA (4, 16, 20, 23, 29). While one integrated copy of the early region is sufficient for full transformation, integrated viral DNA is frequently found to accumulate in full and partial tandem repeats. Repeats are ubiquitous in SV40-transformed mouse fibroblasts and are also seen to a lesser extent in SV40-transformed rat fibroblasts (3, 4, 9, 14, 27).

The capacity of SV40 to generate partial and full repeats in mouse cells has significant consequences. Partial and full repeats of the integrated viral DNA encode and result in the expression of super T antigens by duplications of the T-antigen-coding sequence (7, 11, 18, 21). One such super T antigen, with a molecular weight of 100,000 (100K), is ubiquitous in mouse cell lines fully transformed by SV40. It is always accompanied by the 94K wild-type T antigen in mouse transformants (7). The 100K T antigen does not arise in mouse transformants generated by origin-defective SV40 DNA (6, 13). We hypothesized that origin-defective DNA was unable to form SV40 repeats and that this explained the inability of the origin-minus transformants to express 100K T antigen. However, we found that the viral origin was not required for formation of partial repeats of integrated SV40 DNA in mouse transformants. By comparison, integrated DNA repeat formation was too rare in F2408 rat transformants to permit an assessment of the role of the origin in this process for these cells.

MATERIALS AND METHODS

Cells. SV40-transformed mouse and rat cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (Hyclone) in an atmosphere containing 10% CO₂. All cells were shown to be free of pleuropneumonia-like organisms by Hoechst stain.

DNA transfection. The calcium phosphate method was used for DNA transfection into F2408 or NIH 3T3 cells as described elsewhere (25, 26, 31). Before transfection, the DNA was linearized with a one-cut restriction enzyme. To generate transformants, NIH 3T3 or F2408 cells were transfected with either pSVR1, a plasmid containing wild-type SV40 DNA, or with pSVR1-ori⁻, a plasmid containing SV40 DNA with an altered replication origin that will not replicate

in monkey cells (6). In both plasmids, SV40 DNA and pBR322 DNA were joined at their *EcoRI* sites (Fig. 1) and were linearized with *EcoRI* before transfection. Five transformants each from pSVR1 and pSVR1-ori⁻ transfections were selected as dense foci (6). The DNA from each of these transformants was extracted, digested with *TaqI*, and examined by Southern blot (28, 30) analysis.

Immunoprecipitation. [³⁵S]methionine-labeled cell extracts were prepared and immunoprecipitated with either normal hamster serum or hamster anti-SV40 tumor serum. The proteins were analyzed on 10 to 20% sodium dodecyl sulfate-polyacrylamide gradient gels as described elsewhere (7).

Analysis of the integrated DNA. Genomic digests and Southern blotting were carried out by methods described previously (3, 28, 30). Probes were ³²P labeled by nick translation of pBR322 or SV40 DNA (24). Restriction enzymes were used according to the instructions of the vendor.

RESULTS

SV40 and pBR322 DNA in NIH 3T3 mouse transformants. Transformants isolated after transfection with the origin-defective SV40 DNA contained at least as many subgenomic fragments of integrated SV40 DNA as did transformants made with wild-type SV40 DNA (Fig. 2A). Figure 2A does not reveal whether the multiple *TaqI* bands of SV40-containing DNA in the five origin-defective transformants were the result of multiple insertions or of a postintegration event such as recombination or abortive replication from a host origin. However, the results shown in Fig. 2A do establish the fact that for SV40 in mouse cells, unlike polyomavirus in rat cells, a functional origin is not prerequisite to the appearance of multiple viral inserts.

Since excision of the viral genome has been demonstrated in mouse cells (15), lack of a replication origin may stabilize integrated SV40 DNA. This possibility is supported by Southern blot analysis of transformed DNA digested with *BamHI* and probed with pBR322 (Fig. 2B). Transformants made with origin-defective DNA contained more inserts of pBR322 DNA than did transformants generated with wild-type DNA (Fig. 2B).

Partial repeat formation. When wild-type SV40 infects a mouse cell, its DNA integrates and rearranges into either complete or partial duplications (2, 3, 4, 5, 9, 10, 14, 19, 27). In rat cells, polyomavirus DNA does not rearrange unless a functional polyomavirus replication origin is present (12, 22).

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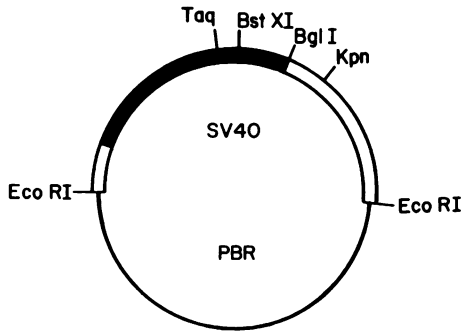


FIG. 1. SV40 DNA used to generate transformants. The plasmid used to generate the SV40 transformants described in this study (6), pSVR1, is as shown, while pSVR1-ori⁻ contains an alteration at the *Bgl*I site in SV40 (6). Both plasmids were linearized at the *Eco*RI site before transfection. PBR, Plasmid pBR322.

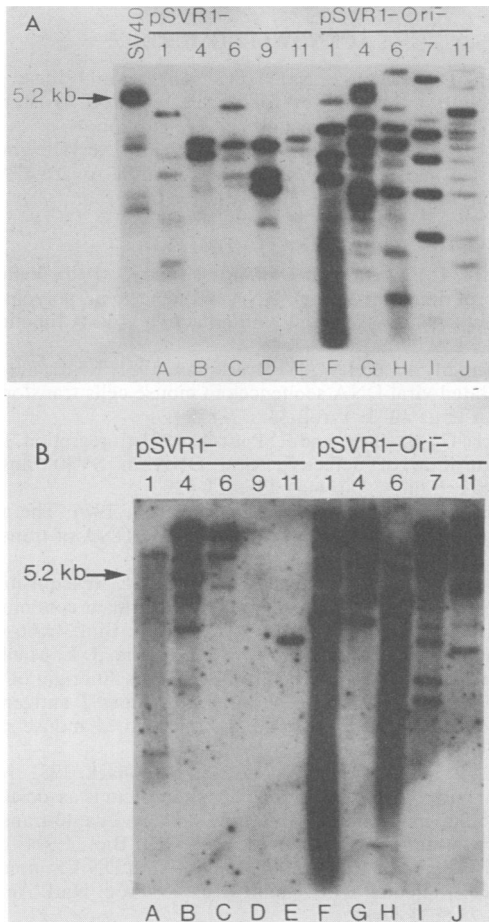


FIG. 2. (A) SV40 and pBR322 DNA in pSVR1 and pSVR1-ori⁻ transformants. High-molecular-weight DNA was isolated from the independently derived transformants indicated above each lane, digested with *Taq*I, and analyzed as described elsewhere (28, 30), with complete SV40 DNA without plasmid sequences as a probe. (B) Cotransfected pBR322 DNA in pSVR1 and pSVR1-ori⁻ transformants. Southern analysis (28, 30) of DNA from cotransfected pBR322 DNA in pSVR1 and pSVR1-ori⁻ transformants with pBR322 as a probe. DNA was digested with *Bam*HI. The arrows indicate the sizes in kilobases (kb) of pSVR1- (A) and SV40 (B).

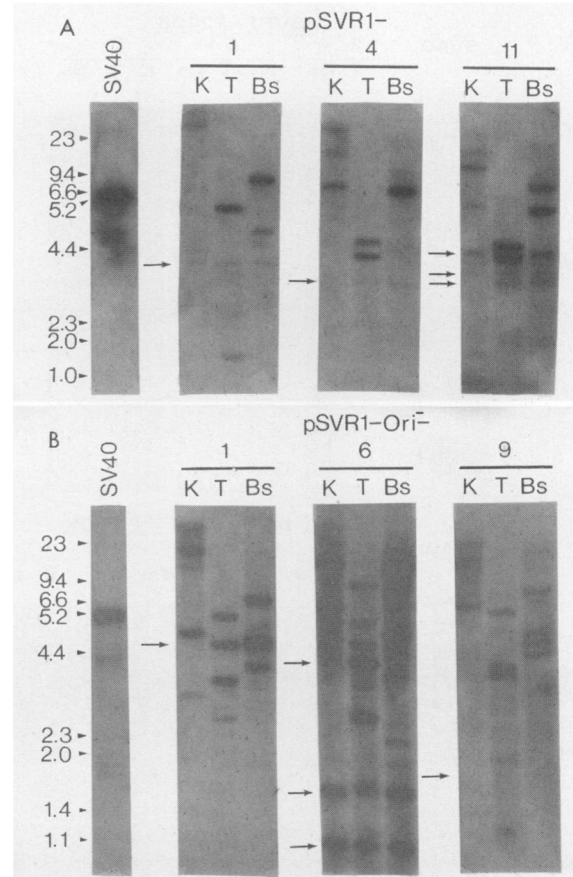


FIG. 3. Detection of partial repeats of viral DNA in pSVR1 (A) and pSVR1-ori⁻ (B) transformants. Tandem repeats of the viral DNA can be identified by digesting the high-molecular-weight transformant DNA with several enzymes which cut the SV40 DNA in one place. The enzymes *Kpn*I (K), *Taq*I (T), and *Bst*XI (Bs) were used (Fig. 1). The numbers and arrowheads on the left are molecular weight markers. The arrows indicate comigration of bands in the separate digests. Each digest is run in a separate, parallel gel lane. Fragments which contain SV40 DNA and comigrate probably represent viral DNA repeats, since a tandem duplication of a set of viral enzyme sites creates fragments of the same size when the DNA is cut at any one of those sites. If the repeat has suffered a deletion between the duplicated enzyme sites, then comigrating fragments will be smaller than the wild-type SV40 DNA length of 5.2 kilobases. Previous reports have determined that about four of five fragments so identified represent partial duplications (3).

Duplications may also arise as a result of a spurt of viral DNA replication initiated at the viral replication origin by T antigen, which has been shown to occur shortly after SV40 enters the cell (8, 17).

When integrated subgenomic-sized SV40 fragments of the same length are detected after digestion of cell DNA by restriction enzymes, these fragments are likely to have originated from tandem repeats of an integrated SV40 sequence (1). In the case of NIH 3T3 transformation with SV40 DNA, we found that partial-repeat formation did not require a functional SV40 replication origin. Mouse cell lines transformed by either pSVR1 or pSVR1-ori⁻ contained partial repeats (Fig. 3A and B). With wild-type transformants, each of three independent subclones revealed one or more subgenomic-length SV40 fragments (Fig. 3A). Identical-sized subgenomic fragments were also observed in each of three origin-minus transfected clones (Fig. 3B). No integrated

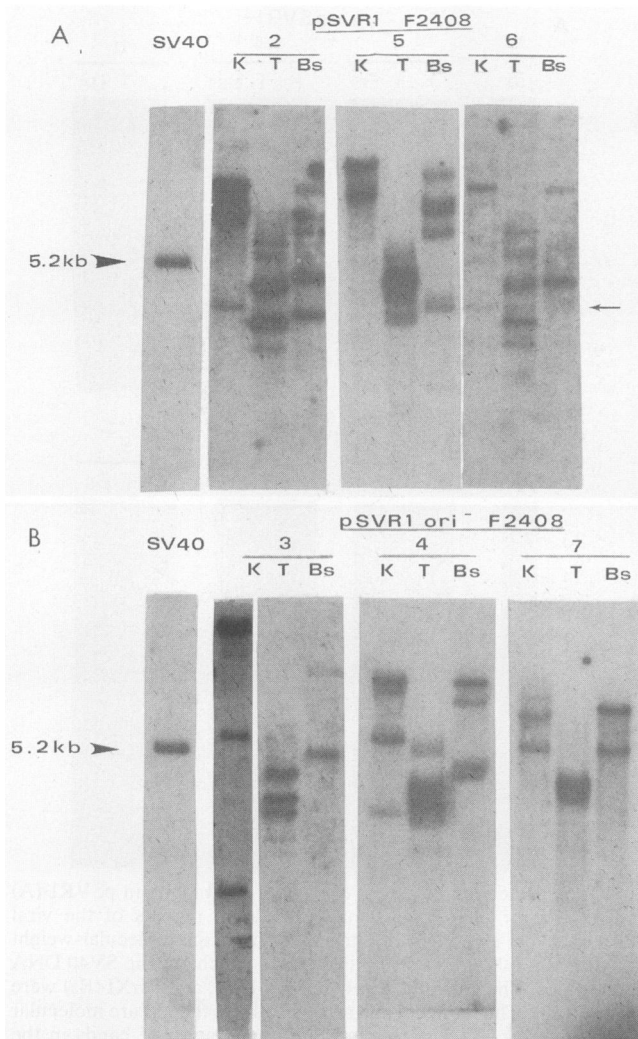


FIG. 4. Analyses of integrated viral DNA in SV40-transformed F2408 cells. Rat clones isolated after transfection by wild-type SV40 (A) and by origin-defective SV40 (B) were analyzed for SV40 sequences as described in the legend to Fig. 3. The size in kilobases (kb) of SV40 is indicated by the arrowhead on the left. The arrow in panel A indicates comigration of bands in the separate digests.

full-length SV40 DNA molecules were detected, possibly because the transformants were derived by transfection with linear DNA, which exposed the ends of each full-length molecule to exonucleolytic degradation.

Viral DNA in SV40-transformed F2408 rat cells. Polyoma-virus-transformed F2408 rat transformants require a functional polyomavirus replication origin for tandem-repeat formation (1, 12, 22). Our results suggest that SV40 DNA in mouse cells behaves differently than polyomavirus DNA in rat cells. To distinguish whether this difference is due to the virus or to the host species, we analyzed SV40-transformed rat F2408 clones (Fig. 4A and B).

Both pSVR1 and pSVR1-ori⁻ transformants had about the same number of SV40 inserts (Fig. 4A and B). Partial repeats were rare in the F2408 transformants in contrast to their occurrence in both types of NIH 3T3 transformants (cf. Fig. 3 and 4). One candidate partial-repeat structure was indicated in clone pSVR1-F2408-6 (Fig. 4A), while none were detected among three pSVR1-ori⁻ F2408 transformants (Fig. 4B).

DISCUSSION

In contrast to origin-minus polyomavirus, origin-minus SV40 has the capacity to form multiple inserts (1–4). In mouse cells, origin-minus SV40 is also capable of generating tandem-repeat insertions. Origin-minus SV40 mouse transformants do not express the 100K super T antigen. The 100K T antigen has been shown by molecular cloning of integrated viral DNA to be coded for by an upstream partial repeat of SV40 early region followed by a full-size early region (18). A 100K-expressing clone is created by linking two appropriately chosen subgenomic SV40 DNA fragments. One of these fragments must contain the N-terminal portion of the early coding region, while the other must consist of a full early region placed downstream of the N-terminal fragment. However, if a similar construction is made by substituting the origin-defective early region for the downstream position, no 100K T antigen is expressed (18). This supports the conclusion of the study presented here that a partial-repeat structure alone is not sufficient to code for the 100K super T antigen. The exact role of the functional origin sequence in coding for this protein is currently under study.

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