

## NOTES

### DNase I Sensitivity of Integrated Simian Virus 40 DNA

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We undertook an analysis of integrated simian virus 40 (SV40) DNA to learn whether the DNase I-sensitive region is retained in the integrated array of mouse transformants. Our results indicate that full-length integrated SV40 chromatin retains a DNase I-hypersensitive region at the same point as in nonintegrated SV40 chromatin. Thus, the lack of a DNase I-hypersensitive region is not likely to be the reason for nonpermissivity of SV40 in mouse cells. In addition, results reported here indicate that a deletion of about 200 base pairs of DNA in the region of the DNase I-hypersensitive site severely reduces the sensitivity of integrated SV40 chromatin. This result is similar to a previously reported result obtained with deletion mutants of SV40 analyzed in the lytic cycle. It is the first report of a DNA lesion affecting DNase I hypersensitivity of a mammalian chromosome.

DNase I sensitivity is currently the most general probe for gene activity in eucaryotic chromatin (8, 25). Frequently, active genes have 5' sites which are hypersensitive to DNase I attack. These sites can be positioned by digestion of isolated nuclei with DNase I and subsequent digestion of the extracted DNA with restriction enzymes. This procedure yields DNA fragments which are bordered on one side by a DNase I-hypersensitive site and on the other side by a known restriction enzyme site. Such fragment sizes, therefore, represent the distance of a restriction enzyme site to a DNase I-hypersensitive site. This analysis has been carried out for the eucaryotic viruses, simian virus 40 (SV40), and polyomavirus (11, 20, 22, 23). Each of these viruses manifests a DNase I-sensitive region, where the 5' termini of both the early late regions begins. This is the region of presumptive promoter sites for gene transcription and of the SV40 72-base-pair repeat, a known transcription enhancer.

SV40 mouse cell transformant SV101 is particularly suited for DNase I analysis of SV40 chromatin because it is one of a few SV40 mouse transformants examined thus far which contains both complete and partially repeated SV40 DNA in the integrated array (2). Partially repeated or defective viral DNA is very common in SV40-transformed mouse cells and is likely to play a role in generating the fully transformed phenotype (1, 11, 22). An analysis of SV101 allows these structures to be studied in the same cell line that contains a complete-length viral genome.

Previous mapping studies of SV101 revealed that it contains one, or a few, large integrated arrays of viral DNA repeated in tandem (2). These arrays have not been mapped from one end to another, but enough data have been obtained to reveal one stretch of viral DNA that is missing ca. 200 base pairs, including the viral *Bgl*I site; one stretch missing 900 base pairs, including the viral *Eco*RI site; one stretch missing 1,500 base pairs, including the viral *Bam* site; and one stretch missing 3,700 base pairs, including both the viral *Eco*RI and *Bam* sites (Fig. 1). Digestion of SV101 DNA with a restriction enzyme which cuts SV40 DNA in one place and Southern blot analysis with SV40 [<sup>32</sup>P]DNA as probe reveals a series of fragments which represent these

deletions and a fragment of 5.2 kilobase pairs (kb) representing the full-length viral DNA.

An *Eco*RI digest of DNA from SV101 chromatin that was treated with DNase I revealed one set of fragments which migrated at about 3.5 kb and another set which migrated at about 1.2 kb (data not shown). These fragments were not seen when naked SV101 DNA was treated with DNase I and digested with *Eco*RI. These preliminary results suggest that the position of the DNase I-hypersensitive site in integrated SV40 DNA was the same as its position in lytic SV40 chromatin (19, 20, 22, 23) (Fig. 1). To be certain of the hypersensitive position in the integrated array, we digested DNA from DNase I-treated nuclei with *Eco*RI and probed the resulting digests with a bacterial plasmid containing either the *Bgl*I-*Eco*RI SV40 late-region fragment or the *Bgl*I-*Bam* early-region fragment (Fig. 1). This procedure manifested a series of DNase I-*Eco*RI fragments which hybridized to the late-region probe (Fig. 2A). They also migrated with, and slightly ahead of, an analogous set of fragments generated by treating free SV40 chromatin obtained from infected BSC-1 monkey cells with DNase I and by subjecting the isolated SV40 DNA to the same *Eco*RI and Southern blot analysis. These SV101 fragments did not appear in SV101 DNA that was not pretreated with DNase I (Fig. 2A). Since many of the SV101 fragments migrated ahead of the fragments isolated from SV40-infected nuclei, it is likely that the DNase I-sensitive site in full-length SV101 viral DNA can frequently extend 50 to 100 base pairs further into the late region than the hypersensitive site of lytic SV40. A complementary analysis with the early region probe (Fig. 2B) reveals DNase I-specific fragments from SV101 which comigrate with early-region fragments released from DNase I-treated SV40 chromatin. In both the early and late probe experiments, the appearance of DNase I-specific fragments was accompanied by the disappearance of the 5.2-kb fragment. These results indicate that full-length viral DNA integrated in SV101 maintains essentially the same DNase I-hypersensitive region found in lytic SV40 chromatin. This result is likely to be dependent on the well-conserved structure of histone proteins which are known to define the DNase I-hypersensitive site in lytic SV40 chromatin (20, 22, 23). Cremisi has conducted a similar analysis of SV40 rat transformant 14B which contains an incomplete copy of the

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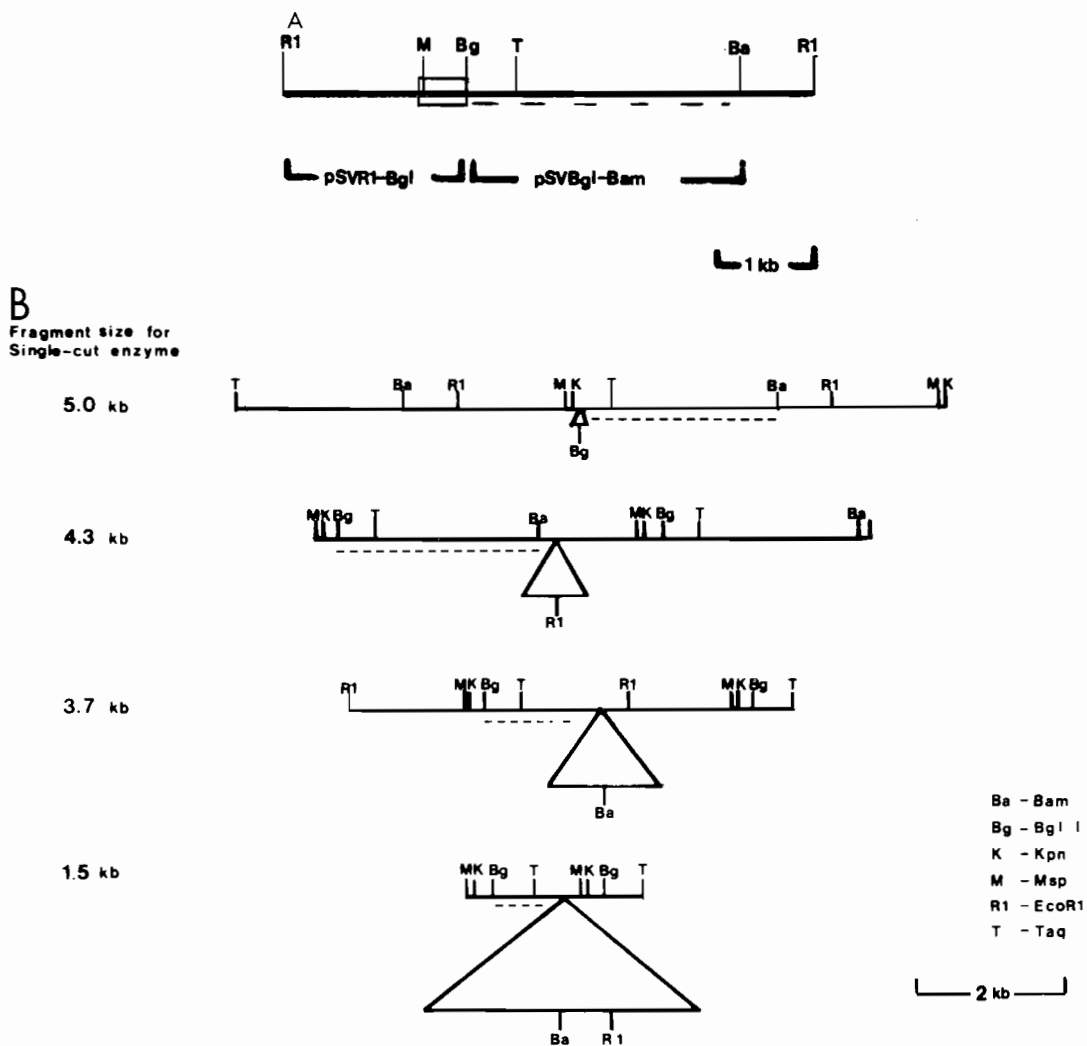


FIG. 1. (A) Schematic representation of SV40 DNA, with the early- and late-region probes used in Fig. 2 indicated as pSVR1-Bgl and pSVBgl-BAM, respectively. The boxed region represents the region of DNase I hypersensitivity found in SV40 chromatin in monkey cells and in SV101. (B) Schematic representation of defective viral DNA found in SV101. These maps were generated by analyzing data presented previously (2). The triangles below the maps indicate regions of SV40 DNA deleted from the surrounding restriction enzyme sites. The single-cut fragment size column numbers are the sizes (in kb) of the SV40 DNA fragment liberated from the stretch of integrated DNA indicated when an enzyme which cuts SV40 DNA one time is used. Although the integrated DNA in SV101 has been mapped well enough to detail the above structures, their order along the chromosome is not known (2). In both (A) and (B), the dashed line represents the viral early region.

viral genome (7). Her results indicate that SV40 DNA integrated in rat cells contains a lytic nuclease-sensitive site as well (7).

It is unlikely that the DNase I-specific fragments of SV101 arise from unintegrated SV40 DNA, which is occasionally seen in SV40-transformed mouse cells. First, digestion of SV101 DNA with an enzyme which does not cut SV40 DNA does not reveal any unintegrated SV40 DNA (2). Second, DNase I-treated SV101 DNA, which is not cut with EcoRI, reveals only high-molecular-weight material hybridizing to SV40 DNA in Southern blot experiments. If any unintegrated SV40 DNA were present in SV101, it would migrate as a 5.2-kb fragment after DNase I digestion (Fig. 2).

An examination of Fig. 2A and B reveals that the 5.0-kb fragment that migrates just ahead of the 5.2-kb fragment is relatively insensitive to DNase I. Thus, after a substantial portion of the 5.2-kb fragment is digested by DNase I (Fig. 2A and B, lanes E) the 5.0-kb fragment remains intact. This

can be explained by the fact that this fragment is missing about 200 base pairs in the region of the viral BglI site. Deletions in this region, and especially of this size, have been shown to reduce or abolish the sensitivity of SV40 chromatin prepared from infected monkey cells (10). Given the good correlation of DNase I sensitivity and transcriptionally active eucaryotic genes, it seems likely that the stretch of SV40 DNA which is proximal to the 200-base-pair deletion in SV101 is inactive (1, 3, 9, 13, 14, 16, 21, 24, 26). In a similar analysis, McGinnis et al. showed that when the *Drosophila* glue protein, Sgs-4, suffered upstream lesions in its DNase I-hypersensitive site, both sensitivity and gene expression were abolished (16).

Anchorage-dependent revertants selected from SV101 lost a great deal of defective viral DNA while still retaining an intact SV40 early region (2). However, anchorage-independent subclones retained most of the defective viral DNA in SV101. These results indicate that defective or partially

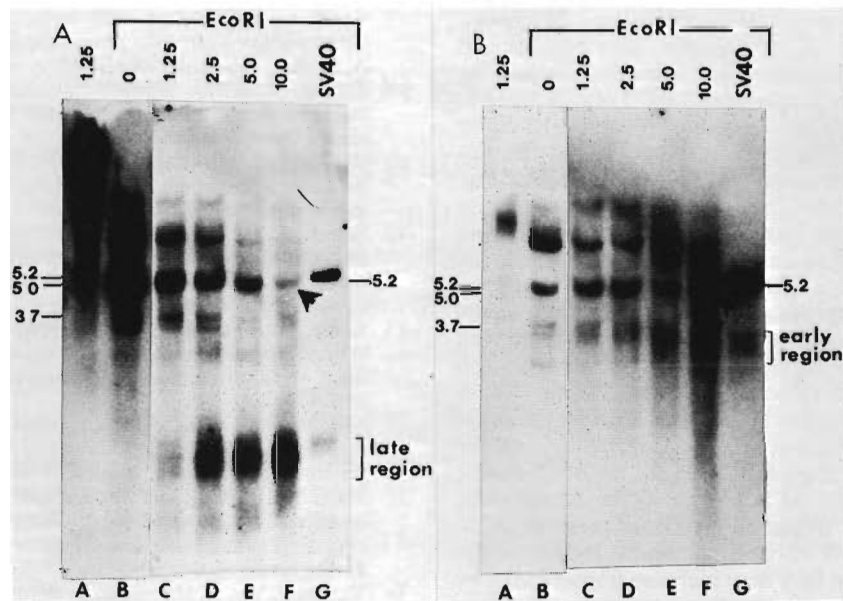


FIG. 2. SV101 nuclei were prepared essentially by the method of Stadler et al. (21). Briefly, SV101 cells were plated on 150-mm dishes at  $3 \times 10^6$  cells per plate 1 day before nuclei preparation. Nuclei were prepared by washing the plates three times with phosphate-buffered saline and by adding 2 ml of RSB lysis buffer (10 mM Tris [pH 7.5], 10 mM NaCl, 3 mM MgCl<sub>2</sub>, and 0.5% Nonidet P-40) per plate. After 10 min at 4°C, nuclei were scraped off the plate and washed three times in RSB buffer without Nonidet P-40. A total of  $0.5 \times 10^7$  to  $1.0 \times 10^7$  nuclei per ml were digested with DNase I at concentrations indicated above each lane, in micrograms per milliliter, for 10 min. The lane labeled SV40 was prepared by digesting SV40-infected BSC-1 monkey nuclei with 50 µg/ml. SV40-infected nuclei were prepared as described above 34 h postinfection. After DNase digestion, nuclei were lysed with 0.5% sodium dodecyl sulfate and digested for 2 h with 500 µg of Sigma type XI protease per ml. DNA was extracted with phenol and chloroform and analyzed by Southern blotting as previously described (2). The arrowhead between lanes F and G points to the fragment at 5.0 kb that is relatively insensitive to DNase I. All fragment sizes indicated are in kb. Lane A was not digested with *EcoRI* before Southern blot analysis.

repeated viral DNA plays a role in generating the anchorage-independent phenotype, either by coding for variant T antigens or by increased gene dosage (4, 5, 12). The distribution of defective viral DNA structures among SV101 subclones is shown in Fig. 3. It can be seen that the 5.0-kb

fragment is present in two of the anchorage-dependent revertants. We propose that this fragment did not segregate according to the anchorage-independent phenotype because it was transcriptionally inactive, as judged by the DNase I sensitivity results described above.

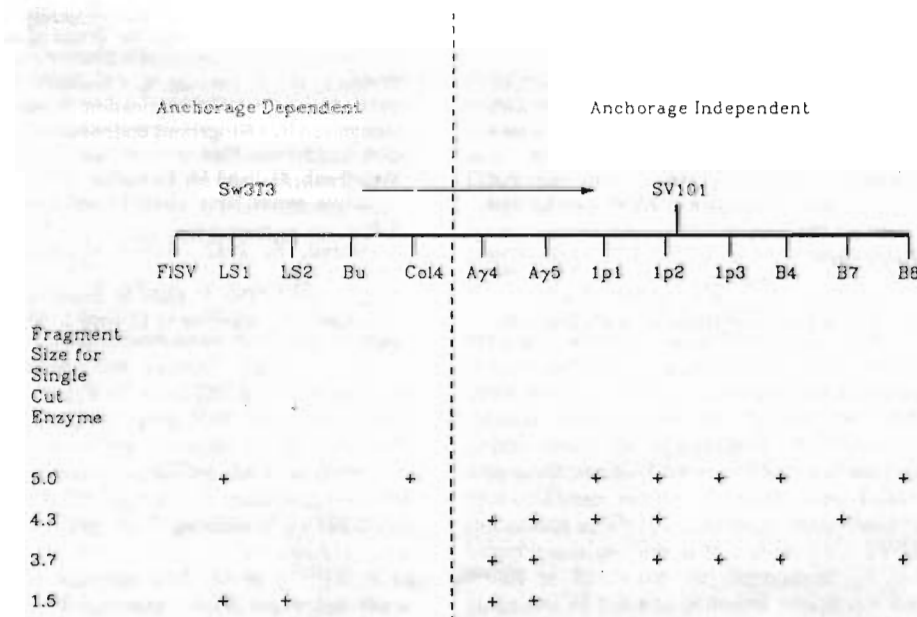


FIG. 3. Cell lineage diagram indicating the loss and retention of defective viral DNA among anchorage-independent and -dependent subclones of SV101. The dotted line divides the anchorage-dependent and -independent subclones. Fragment sizes are in kb.

A prediction of this proposal is that other fragments, which contain deletions of nonhypersensitive regions and segregate according to the anchorage phenotype, would be more sensitive to DNase I than would the 5.0-kb fragment. An example of such a fragment generated by an *EcoRI* digest is the 3.7-kb fragment. Given the assumption that the 5.0- and 3.7-kb fragments are equimolar, an examination of Fig. 2A reveals that the 3.7-kb fragment is more sensitive to DNase I than is the 5.0-kb fragment. (The fate of the 3.7-kb fragment is more difficult to determine in Fig. 2B because the upper range of the DNase-specific fragments liberated from full-length viral DNA appears at about the same point on the gel). Therefore, it is likely that the partial repeats of viral DNA seen in SV40-transformed cells drive the anchorage-independent phenotype through their expression. These partial repeats are frequently involved in the expression of super T antigens, at least one of which correlates with the anchorage-independent phenotype (2, 5, 6, 18, 24). Another possible role for the partial repeats may be to maintain regions of access for transcription complexes. If these regions contain the SV40 72-base-pair repeat located in the hypersensitive site, then they may increase transcription by quite some distance from viral coding sequences (15, 17).

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