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 covalts both complete and partially repeated SV40 DNA in the integrated array (21). 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 BglII site; one stretch missing 900 base pairs, including the viral EcoRI site; one stretch missing 1,500 base pairs, including the viral BamHI site; and one stretch missing 3,700 base pairs, including both the viral EcoRI and BamHI sites (Fig. 1). Digestion of SV101 DNA with a restriction enzyme which cuts SV40 DNA in one place and Southern blot analysis with SV40 [32P]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 EcoRI 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 EcoRI. 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 EcoRI and probed the resulting digest with a bacterial plasmid containing either the BglII-EcoRI SV40 late-region fragment or the BglII-BamHI early-region fragment (Fig. 1). This procedure manifested a series of DNase I-EcoRI 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 EcoRI 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
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 BglII 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 euchromatic 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 eyeless 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 × 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_2, 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 × 10^7 to 1.0 × 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 (22). 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 anchor 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 anchor- 

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LITERATURE CITED


5. Chen, S., M. Verderame, A. Lo, and R. Pollack. 1981. Nondylic simian virus 40-specific 100K phosphoprotein is associated with anchor- 


12. Hiscott, J., P. Murphy, and V. Defendi. 1980. Amplification and rearrangement of integrated SV40 DNA sequences accompany the selection of anchor- 


22. Varshavsky, A. J. 1979. A stretch of late SV40 viral DNA about 400 bp long which includes the origin of replication is specifically exposed in SV40 minichromosomes. Cell 16:45–466.


