

DNA Rearrangement and the Role of Viral Origin in SV40-transformed Mouse Cells

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In simian virus 40 (SV40)-transformed mouse cells, a 100-kD super T antigen is always found in addition to the wild-type 94-kD and 17-kD T antigens. We have determined the coding sequence for this super T antigen, which includes two separate partial repeats of the SV40 genome. The downstream repeat contained the complete coding sequence for the SV40 large T antigen, whereas the upstream repeat was a truncated copy of the same gene, which varied in length in different clones.

We also have shown that in SV40-transformed mouse cells, a functional origin of replication is not needed for either partial repeat formation or subclonal rearrangement of integrated viral DNA.

Simian virus (SV40) encodes two early proteins, large T and small T antigens, with molecular masses of 94 kD and 17 kD, respectively (Tooze 1981). Both proteins are derived from a single transcript whose 5' end lies near the viral origin of replication.

The large T antigen has been shown to be involved in transformation, in some cases including maintenance of certain transformed phenotypes in nonpermissive cells (Tooze 1981). T antigen also participates directly in many biochemical events, including initiation of viral DNA replication in lytically infected permissive cells (Tegtmeyer 1975), down-regulation of early RNA synthesis (Tegtmeyer et al. 1975; Reed et al. 1976), activation of host ribosomal genes (Soprano et al. 1980), stimulation of cellular DNA synthesis (Henry et al. 1966), provision of adenovirus helper function (Cole et al. 1979), high-affinity binding to specific DNA sequences at the SV40 origin of replication (Tjian 1978), *in vitro* ATPase activity (Tjian and Robbins 1979), and formation of a stable complex with a cellular 54-kD phosphoprotein (Lane and Crawford 1979).

Infection of rodent cells by SV40 frequently results in the integration of tandem arrays of viral DNA into host chromosomes (Bender and Brockman 1981; Clayton and Rigby 1981; Blanck et al. 1982). A comparison of the amplified arrays of viral sequences in several SV40 mouse transformants reveals that they share some consistent, structured features. Usually they contain one or more copies of a full-length viral genome, in addition to several partial copies (Bender and Brockman 1981; Clayton and Rigby 1981; Sager et al. 1981; Blanck et al. 1982). Generation of variant-size T antigens probably occurs through viral DNA rearrangements since tandem arrays of SV40 DNA encode and express variant-size T antigens (Chang et al. 1979; Kress et al. 1979; Smith et al. 1979; McCormick et al. 1980; Chen et al. 1981). Those that are larger than 94 kD T are termed "super T antigens" (Chang et al. 1979; Kress et al. 1979; Smith et al. 1979; McCormick et al. 1980; Chen et al. 1981).

One such super T antigen, 100 kD, is ubiquitous in cell lines transformed by either SV40 virus infection or SV40 DNA transfection. This 100-kD T antigen does not appear in mouse transformants generated by a variant SV40 DNA virus that lacks a functional viral replication origin (Chen et al. 1983). This is true for two different mutants; one has a 6-bp in-phase deletion (Gluzman et al. 1980), and the other has a 4-bp insertion at the origin of replication (Chen et al. 1983).

SV40 DNA Rearrangement

Multiple copies of foreign DNA that are introduced into a cell by infection, transfection, or microinjection often integrate in a tandem array, and significant perturbations occur in the host sequences adjacent to sites of integration (Botchan et al. 1980; Stringer 1982; Kopchick and Stacey 1984). The input foreign DNA can be of either supercoiled or linear form and still be efficiently ligated together in head-to-tail polymers (Wilson et al. 1982). Although these DNA concatemers are relatively stable, subsequent rearrangements also take place.

Rearrangements of SV40 DNA can occur both before integration and after integration. Chia and Rigby (1981) have demonstrated that prior to integration SV40 replicates in the form of large polymers and that double-crossover events can account for some multiple insertions of viral DNA. However, further amplification, excisions, and recombinations also occur after the initial integration event.

Rearrangements of SV40 DNA have been reported in many mouse transformants (Bender and Brockman 1981; Clayton and Rigby 1981; Blanck et al. 1982). We have shown that selection for phenotypic reversion from the transformed state to a more normal serum or anchorage-dependent state is accompanied by rearrangement of integrated SV40 DNA. Anchorage-dependent revertants preferentially lose defective viral DNA while retaining an intact SV40 early region and the ability to

express lytic-size large and small T antigens. A considerable amount of viral DNA rearrangement also occurs in some anchorage-dependent revertant cell lines, such as the line LS₁ isolated from the fully transformed mouse line SV101. In semisolid medium, anchorage-independent "relapsed" subclones can be isolated from LS₁. These have undergone amplification of their integrated viral DNA inserts (Blanck et al. 1982). Similar results were reported in rat transformants: Rat transformants selected for growth in semisolid medium contain amplified viral sequences, while those isolated from non-selective growth only contain a single insert (Mougueau et al. 1980).

In SV40-transformed mouse cells, DNA rearrangements also occur with serial cell passage in culture. Recently, we and others (Sager et al. 1981; Chen et al. 1983) have shown that the SV40-transformed BALB/c-3T3 cell line SVT2 contains only one variant early region of SV40, about 4.4 kb in length. The only detectable virus-specific protein made by this region is a 100-kD super T antigen. Upon subcloning or passaging in culture, a normal-size early region of 2.7 kb and lytic-size 94-kD large T and 17-kD small T antigens arise in SVT2 cells in addition to the super T antigen (Chen et al. 1983).

SV40 origin of replication is not required for DNA rearrangements

It has been assumed that SV40 DNA rearrangement depends on a functional viral origin of replication, because this has been shown to be the case for polyoma rat transformants (Basilico et al. 1979; Pellegrini et al.

1984). However, subclones derived from mouse transformants generated either with wild-type or origin-defective SV40 show DNA rearrangements (Fig. 1). This is in direct contrast to results obtained from polyoma rat transformants. In the case of SV40, mouse transformants can have partial repeats of SV40 DNA and DNA rearrangements can occur, despite the absence of a functional viral origin of replication (G. Blanck et al., in prep.). The difference in the two results may be due to the differences in host cells and/or virus types.

The coding region of super T antigens

Some super T antigens are encoded by variant early region sequences that contain internal in-phase duplications. For instance, the sequence that encodes a 145-kD super T antigen in mouse transformants contains a perfect, in-phase duplication of 1212 bp within the large T second exon from nucleotides 4103 to 2892 (Lovett et al. 1982). Similarly, May et al. (1981) have determined that the template sequence for a 115-kD super T antigen in rat transformants includes a duplication of 572 bp in the same region (4116-3544). The duplicated sequences are not adjacent, however; they are separated by a 93-bp segment that is a nearly perfect inversion of a sequence located within the large T intron.

Two other super antigens from mouse transformants contain internal duplications that span the acceptor splice junction between the large T intron and the second exon. The 130-kD super T antigen in mouse transformants contains a duplication of 788 bp, which extends from nucleotides 4588 to 3800 (Lovett et al. 1982). The 100-kD super T antigen in SVT2 has a very

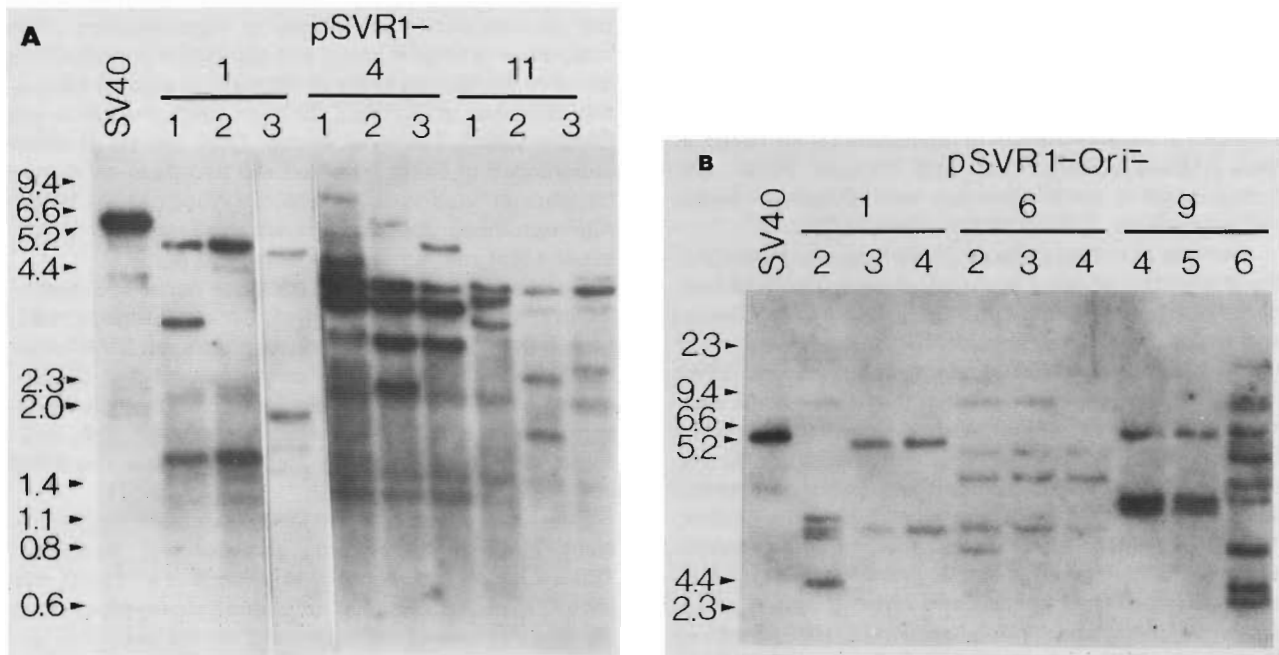


Figure 1 Subclonal rearrangement of viral DNA in pSVR1 (A) subclones: 1-1, 1-2, 1-3, 4-1, 4-2, 4-3, 11-1, 11-2, and 11-3 and pSVR1-ori⁻ (B) subclones: 1-2, 1-3, 1-4, 6-2, 6-3, 6-4, 9-4, 9-5, and 9-6 transformants. High-molecular-weight DNA was extracted from the subclones, digested with *Taq*, and probed with ³²P-labeled SV40 DNA. Comparison of the subclones indicates the rearrangement of integrated SV40 DNA.

large duplication of 1750 bp that also spans the acceptor splice junction (Sager et al. 1981). It is still not known whether the duplicated part of the intron is used as a coding sequence or a new splice pattern is involved.

Both the 115-kD and 145-kD super antigens have been cloned and transfected into rodent and monkey cells, and both of these super T antigens are able to transform rodent cells, but neither one is able to support lytic replication (May et al. 1981; Clayton et al. 1982; Lovett et al. 1982).

The 100-kD super T antigen

We have been studying the 100-kD super T antigen that is found in fully transformed, anchorage-independent mouse cell lines and lost specifically in subclones that have regained serum and/or anchorage requirements (Chen et al. 1981).

We have identified the coding sequences for the 100-kD super T antigen in two different SV40 mouse transformants (SV101 and SV3T3 CIM). Unlike the other super T antigens, the 100-kD T antigen is not coded by internal duplication of SV40 DNA. In both clones, one complete SV40 early region is preceded by a truncated copy of the early region (Levitt et al. 1985). The truncated early region can be of different lengths but includes the first exon, the intron of large T antigen, and part of the second exon. The full-length early region can be separated from the truncated early region by as much as 600 bp as in pSV3T3-M-A, or it can be im-

mediately adjacent as in p100D. pSV3T3-M-A was one of the clones from a library of genomic clones of SV3T3 CIM made by Clayton and Rigby (1981). Although p25B was made from SV101 after virus rescue by fusion with monkey cells, p100D was constructed in vitro from wild-type SV40 by placing a truncated copy of the early region upstream from a full-length early region.

All three plasmids when transfected into monkey cells express the 100-kD super T antigen (Fig. 2). Both p25B and pSV3T3-M-A have been mapped extensively with restriction enzymes. The upstream, truncated early region is of two different sizes; however, both contain a complete SV40 early region downstream (Fig. 3). The truncated early region in both cases starts before the origin/control region and continues past the acceptor splice site for the large T antigen. Only about 100 bp of mouse DNA separate the two inserts in p25B, whereas in pSV3T3-M-A about 600 bp of host DNA are present between the upstream partial repeat and the downstream early region.

For the 100-kD super T antigen to be transcribed, the two insertions must be colinear; that is, if the partial repeat upstream is separated from the complete early region, then only wild-type 94-kD T antigen is produced. Therefore the partial and full-length early regions must be *cis* for 100-kD super T antigen expression (Levitt et al. 1985).

Mouse transformants generated from origin-defective SV40 fail to express the 100-kD super T antigen (Chen et al. 1983). Partial repeats of integrated SV40 DNA occur in these transformants as examined by three

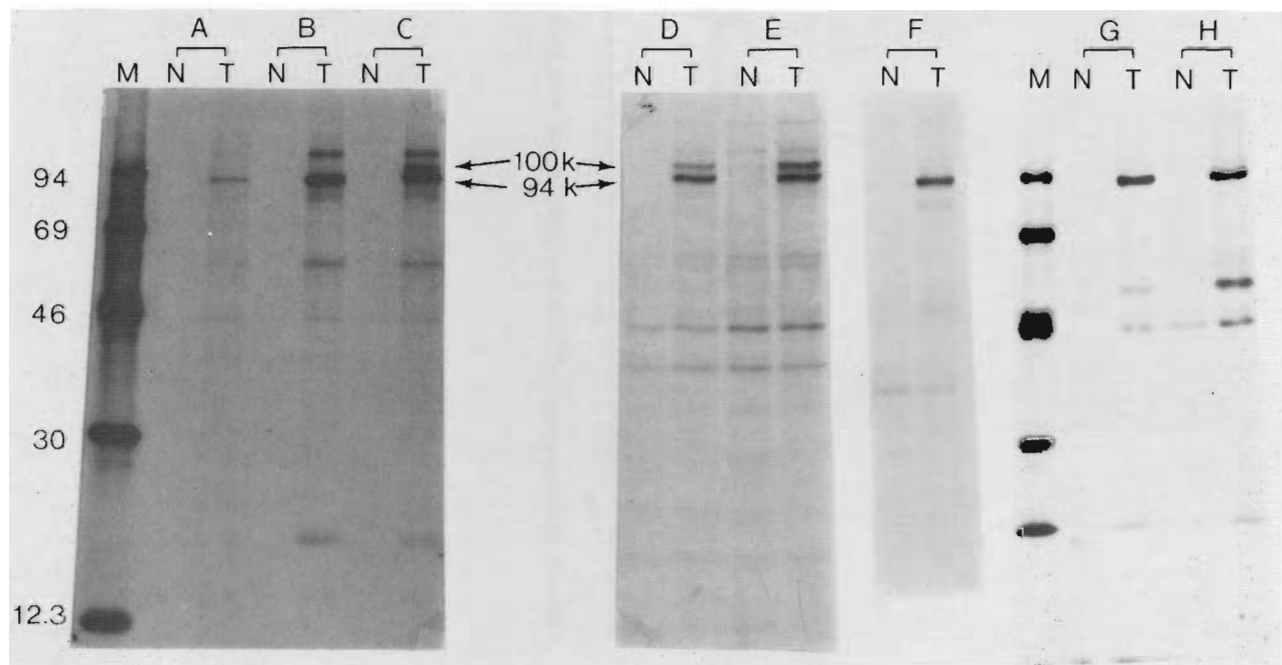


Figure 2 Immunoprecipitation of viral proteins in [35 S]methionine-labeled monkey cell extracts at 48 hr posttransfection with the following plasmid DNA and analyzed on a 10–20% SDS-polyacrylamide gel: wild-type SV40 pSVR1 (A), origin-defective SV40 pSV *ori*⁻ (B), p25B (C), pSV3T3-M-A (D), p100D (E), p25B-*ori*⁻ (F). Lanes G and H are [35 S]methionine-labeled cell extracts from mouse transformants generated by cDNA clones of large T pSVT-2 (lane G) and pSVT-5 (lane H).

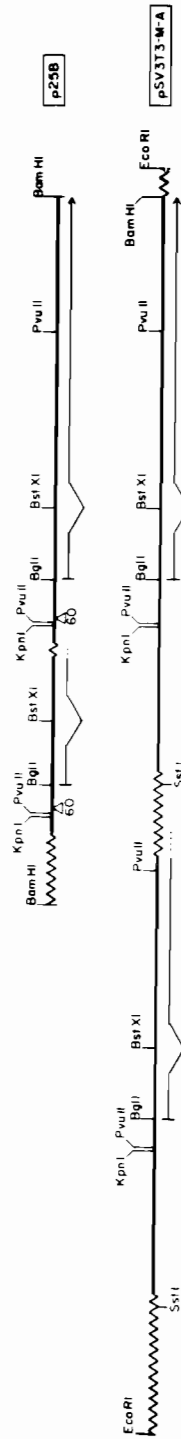


Figure 3 Restriction maps of p25B and pSV3T3-M-A. Viral DNA is indicated by solid straight lines, and host DNA is indicated by sawtoothed lines.

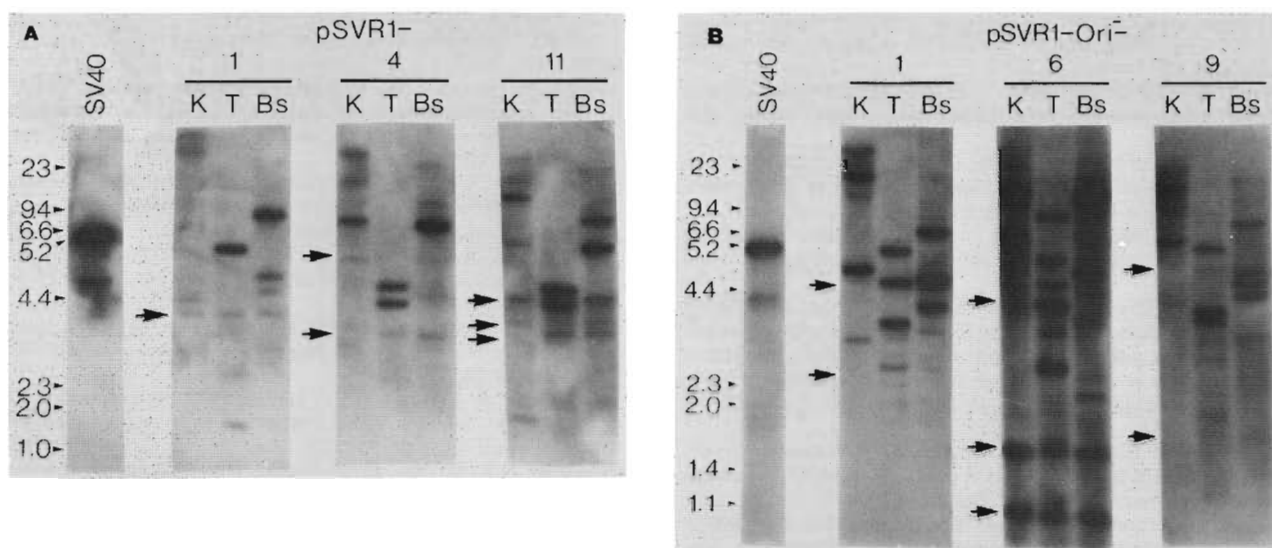


Figure 4 Partially repeated viral DNA in pSVR1 and pSVR1-ori⁻ mouse transformants. Tandem repeats of the viral DNA can be identified by digesting the high-molecular-weight transformant DNA with several enzymes that cut the SV40 DNA in one place. Each digest is run in a separate, but parallel, gel lane. Fragments that contain SV40 DNA and comigrate most likely represent viral DNA repeats, since a tandem duplication of a set of viral enzyme sites creates the same size fragments when the DNA is cut off 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 kb. Although it is possible that fragments detected in this way represent some other structure, more-detailed work has shown that ~80% or more of the fragments so identified do in fact represent partial duplications (Blanck et al. 1982). Here, the enzymes *Kpn* (K), *Taq* (T), and *Bst*XI (Bs) were used. The arrowheads indicate comigration of bands in the separate digests: (A) pSVR1 transformants; (B) pSVR1-ori⁻ transformants.

one-cut restriction enzymes (Fig. 4). Thus, formation of tandem arrays of integrated SV40 can occur in the absence of a functional origin of replication.

If the downstream, full-length SV40 early region is replaced by the origin-defective SV40, then no 100-kD super T expression is seen (Fig. 2, lane F). The sequences at the origin of replication in the downstream full-length region are crucial for the 100-kD super T antigen expression. The origin may serve as a splice signal rather than coding region for the 100-kD protein.

Either one or both of the acceptor and donor splice sites of the large T antigen are needed for the generation of 100-kD T antigen. Mouse transformants generated only by the cDNA clone of large T antigen express the wild-type 94-kD antigen even after many passages in culture (Fig. 2, lanes G and H). Small T antigen deletion mutant 884 generated mouse transformants that express the 100-kD super T antigen (Chen et al. 1983).

The transcripts for the wild-type 94-kD and 17-kD antigen probably initiate at the downstream, full-length early region, whereas transcription for the 100-kD super T antigen probably initiates at the partial repeat upstream and continues through the downstream, full-length early region. The primary transcript is then removed by splicing to generate the 100-kD super T antigen.

Since the 100-kD super T antigen is ubiquitous in SV40-transformed mouse cells, we would like to directly test the functions of this protein. This will only become possible when we obtain a clone that produces the 100-kD super T antigen exclusively.

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