Conversion Through Homologous Recombination of the Gene Encoding Simian Virus 40 115,000-Molecular-Weight Super T Antigen to a Gene Encoding a Normal-Size Large T Antigen Variant

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We have previously cloned the gene encoding a 115,000-Mr super T antigen (115K super T antigen), an elongated form of the Simian virus 40 large T antigen, originating from the rat cell line V 11 F1 clone 1, subclone 7 (May et al., J. Virol. 45:901–913, 1983). DNA sequence analysis has shown that the 115K super T antigen gene contains notably an in-phase duplication of a sequence located in the region of tsA mutations. We have also shown that the 115K super T antigen gene is able to induce the formation of transformed foci in transfected rat cells. After rat cell cultures were transfected with the cloned gene encoding 115K super T antigen, we obtained a large number of transformants as reported in this paper. In these transformants, we detected a very high frequency of new T antigen variants, as shown by immunoprecipitation of the cell extracts with anti-Simian virus 40 tumor serum followed by electrophoresis in sodium dodecyl sulfate-polyacrylamide gels. Based on these results and all of the data presently available, it appears likely that the input plasmid or cosmid DNAs containing the cloned gene were first subjected to recombination events that yield new variant T antigen genes before these recombinant genes become integrated. The new variant T antigens observed in the transformants were predominantly those comigrating with normal-size large T antigen. In fact, these latter variants appeared to be indistinguishable from wild-type large T antigen as judged by restriction mapping by Southern blotting of the total genomic DNA of the transformants. Models of intermolecular or intramolecular homologous recombination occurring between or within the input plasmid or input cosmid DNA molecules are proposed to account for the formation of such revertants.

Large chromosomal rearrangements have been found in many living organisms, notably in eucaryotes. These changes in chromosome structure (rearrangements) include rearrangements, amplifications, deletions, and translocations of DNA segments. Such rearrangements are involved (i) in the genome evolution (11), (ii) in the rearrangements of mammalian antibody genes during lymphocyte development (36), (iii) in the changes, mediated by mobile elements, in gene order and expression (36), and (iv) in the genesis of B cell-derived tumors in mice and humans (19).

Similarly, after infection by simian virus 40 (SV40) or its DNA transfection into cultured mammalian cells, the SV40 genome frequently undergoes mutations and recombinations in these cells (5, 30, 38, 41, 42, 48, 49).

In SV40-transformed rat or mouse cell lines, rearrangements of viral and adjacent cellular sequences occur during or after integration (2, 4, 9, 16, 26, 28, 34). The altered templates encode either shortened forms (truncated T antigens [7]) or elongated forms (super T antigens [8, 23, 25]) of large T antigen. The templates for truncated T antigens are interrupted in the distal exon of the SV40 early region, whereas the templates for super T antigen frequently contain in-phase tandem duplications of the tsA region (23, 25).

We previously cloned (25) the gene encoding a 115,000-Mr, super T antigen (115K super T antigen) which was originally found in the rat cell line V 11 F1 clone 1, subclone 7. DNA sequence analysis showed that the 115K super T antigen gene contained notably an in-phase duplication of a sequence located in the region of tsA mutations. We also showed (26) that the 115K super T antigen gene is able to induce the formation of transformed foci in transfected rat cells. The focus-derived cell lines that were tested appeared to produce 115K super T antigen. On that basis, we showed that 115K super T antigen retains the transforming capacity of large T antigen (26).

Following up this observation, we have made a more extensive study, reported in this paper, of a larger number of transformants derived from rat cell cultures after transfection with the cloned gene encoding 115K super T antigen. A striking new feature has emerged from this study. It appears that the transfecting super T antigen gene is subject to recombination events in rat cells, generating, with a high frequency, a new gene(s) encoding normal-size variants of large T antigen. From the experimental results described below, we have hypothesized that the recombination events in these rat cells are homologous.

MATERIALS AND METHODS

SV40 nucleotide sequence numbering system. Nucleotides in the SV40 genome are numbered according to the system proposed by Reddy et al. (31) modified to take into account 17 base pairs (bp) that were previously overlooked (43). Nucleotides in pBR322 are numbered by the system of Sutcliffe (39).

Cosmid and plasmids. The structures of the plasmids and cosmid used in this study are shown in Fig. 1. The recombinant cosmid SVE 5 Kb was constructed by May et al. (25). This cosmid contains the entire 115K super T antigen gene derived from the genome of V 11 F1 clone 1, subclone 7 cells (25). We constructed a plasmid, pSvST, by inserting a fragment into the BamHI site of plasmid pBR322. This fragment was similar to the BamHI-cleaved linear SV40 except that the authentic SV40 early region was replaced by the gene for super T antigen (26).

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Plasmid psT was derived from plasmid pSVsT. After digestion of pSVsT with EcoRV, the large fragment of this plasmid, extending from the unique EcoRV site in SV40 at nucleotide 708 to the unique EcoRV site in pBR322 at nucleotide 189, was isolated and then recircularized, yielding plasmid psT. The cosmid and all of the plasmids mentioned above contain the gene encoding 115K super T antigen. Plasmid pLP was derived from plasmid pSV1 (3) which contained the entire early gene segment (as the large BamHI-HpaII fragment of SV40) cloned into the EcoRI site of pBR322. Plasmid pSV1 was digested with KpnI, which cleaved the plasmid at nucleotide 234 in SV40, and with BamHI, which cleaved the plasmid at nucleotide 375 in pBR322. The large fragment obtained by this double digestion was ligated with the SV40 genome fragment, corresponding to the SV40 late region, obtained by double digestion with KpnI (unique cleavage site at nucleotide 234) and BclI (unique cleavage site at nucleotide 2706). Note that plasmid pLP contains the complete authentic SV40 genome and that the terminal sequences of the viral insert, consisting of the small fragment extending between the restriction sites of BamHI and BclI in SV40 (13), are repeated.

All the plasmids and the cosmid used here were derived from plasmid pBR322 and contained the “poison sequence” of pBR322 (24).

The Escherichia coli K-12 strains used for transformation (and into which plasmids or the cosmid was introduced) were 1106 (su2 su3 hsdR hsdM) and HB101 (pro leu thi lacY hsdR endA recA rpsL20 ara-14 galK2 xyl-5 mtl-1 supE44).

For the preparation of the DNA of the cosmid and plasmids, the transformed bacteria were first grown in L broth overnight at 37°C (without addition of chloramphenicol), and then the DNA was purified by the cleared lysate.
technique of Clewell and Helinski (10) followed by ethidium bromide-cesium chloride equilibrium gradients. The structures of the plasmids and the CsCl used for transfection experiments were verified by analysis with the appropriate restriction enzymes.

Transformation with recombinant DNA. Secondary cultures of rat kidney cells were grown in petri dishes (6-cm diameter) in Dulbecco modified Eagle essential medium (Gibco Laboratories) containing 10% fetal calf serum (Gibco). Transfections were performed by the method of Wigler et al. (47) with some modifications. When the cells reached a stage of 70 to 80% confluency, the medium was replaced with Dulbecco modified Eagle essential medium supplemented with 2.5% newborn calf serum and 2.5% fetal calf serum (Gibco). After 4 h, the precipitated DNA in HEPES buffer (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; containing 5 μg of recombinant DNA, 15 μg of calf thymus carrier DNA, and calcium chloride to a 125 mM final concentration, was added to the cultures without removal of growth medium. The medium was changed after 20 h at 37°C and, thereafter, once weekly. Dishes were scored for foci after 3 to 4 weeks, and a number of foci, randomly selected, were grown up individually to a cell line. (Each focus and the cell line derived from it were labeled with the same number.)

Antisera and monoclonal antibodies. The anti-SV40 tumor serum used was a pool of sera obtained from tumor-bearing Syrian hamsters which had been inoculated with SV40-transformed TSV5 clone 2 hamster cells (26). Control serum was a pool of sera obtained from normal adult Syrian hamsters. The mouse hybridoma lines PAb405, PAb414, PAB416, and PAB419 (originally lines L5, L14, and L19) were described by Harlow et al. (15). The cell culture media of these hybridomas were used as the source of monoclonal antibodies specific for SV40 large T antigen.

Radiolabeling and extraction of cells; immunoprecipitation, electrophoresis, and autoradiography of labeled proteins. The transformed cell lines were seeded at 5 × 10⁶ cells per petri dish (10-cm diameters, Corning Glass Works). Forty-eight hours after seeding, the cultures were incubated for 1 h in methionine- or phosphate-free Eagle minimal essential medium and then labeled for 3 h either with 25 to 50 μCi of L-[³⁵S]methionine per ml (750 to 930 Ci/mmol; Radiochemical Centre, Amersham, England) or with 300 μCi of ³²P,PO₄ per ml (C.E.A.) in methionine- or phosphate-free medium.

In some experiments, confluent cultures of primary baby mouse kidney (BMK) cells were prepared and infected with SV40 as previously described (20). SV40-infected BMK cells were labeled at 24 h after infection with the same radioactive molecules and under the same conditions used for transformed cells.

After being labeled, the cells were scraped, washed twice with ice-cold phosphate-buffered saline, and suspended at a concentration of 5 × 10⁶ cells per ml in Tris-buffered saline (pH 9) containing 0.5% Nonidet P-40 (Shell Chemical Co.), 10% glycerol, and 2 mM diisopropyl-fluorophosphate (Serva) as a protease inhibitor. After 20 min at 4°C, the suspension was centrifuged for 30 min at 30,000 × g. The supernatant was brought to 1 mg/ml in bovine serum albumin-0.5% Triton X-100-0.06% sodium dodecyl sulfate (SDS)-0.5% sodium deoxycholate, and then the portion corresponding to one dish was incubated with 2 μl of hamster anti-SV40 tumor serum or control hamster serum or with 200 μl of culture medium from monoclonal antibody-producing clones and 20 μl of settled protein A-Sepharose CL4B (Pharmacia Fine Chemicals, Inc.) as described by Schwyzer et al. (35). The suspension was gently agitated overnight at 4°C and then poured into a column in which the Sepharose formed a layer 1 mm or more in thickness. The Sepharose beads were washed three times with 0.2 ml of buffer containing 0.1 M Tris-hydrochloride (pH 8.8), 0.5 M LiCl, and 1% 2-mercaptoethanol. Immune complexes were then eluted from the Sepharose beads with 50 μl of electrophoresis buffer containing 0.08 M Tris-hydrochloride (pH 6.8), 2% SDS, 5% 2-mercaptoethanol, 15% (wt/vol) glycerol, and 0.001% bromophenol blue and incubated at 100°C for 10 min before analysis by SDS-polyacrylamide gel electrophoresis as described by Laemmli (21). Acrylamide gels (7.5%) were used. Electrophoresis was carried out at room temperature at 30 mA for 3 to 4 h. The gels were stained with Coomassie brilliant blue, destained, dried, and exposed to Kodirex X-ray films, usually for 2 to 4 days. The following molecular weight standards were used to calibrate the gels: 115K super T antigen, phosphorylase a (97,000), SV40 large T antigen (90,000), bovine serum albumin (68,000), and catalase (60,000); p53 protein, aldolase (40,000).

DNA extraction and restriction enzyme analysis by blot hybridization. High-molecular-weight cellular DNA was isolated from transformed cells as described previously (25). This DNA was doubly digested either with TaqI and PstI or with TaqI and HindIII restriction endonucleases.

Digested cell DNA (15 μg) was loaded into a slot in a vertical slab gel in a buffer (electrophoresis buffer) containing 40 mM Tris-hydrochloride (pH 7.8), 5 mM sodium acetate, and 4 mM EDTA. One adjacent slot contained a digested mixture of SV40 DNA and BMK cell DNA (7 × 10⁻² and 15 μg, respectively); another slot contained a digested mixture of SVE 5 Kb cosmid DNA and BMK cell DNA (15 × 10⁻² and 15 μg, respectively); these mixtures were digested before electrophoresis, either with TaqI and PstI or with TaqI and HindIII restriction enzymes. Electrophoresis was performed at 2 V/cm for 14 h. DNA was transferred from the gel to a nitrocellulose sheet (Schleicher & Schuell, Inc.) as described previously (12). Hybridization of the ³²P-labeled DNA probe (see below) to the DNA that was immobilized on the membrane filter and the washing of the filter was carried out as previously described (12).

The DNA probe used in this study consisted of the TaqI-PstI fragment of SV40 DNA that extends from nucleotide 4675 to nucleotide 3144 (Fig. 2). SV40 DNA was doubly digested with TaqI and PstI restriction endonucleases, and the appropriate 1,352-bp fragment was purified by two successive fractionations through sucrose density gradients. The nick translation of purified DNA fragment (1 × 10⁶ to 2 × 10⁶ cpmp/μg) was performed as reported by Rigby et al. (32).

RESULTS

The transforming activity of the gene for 115K super T antigen was tested by transfecting recombinant DNA (pSvSt, pSt, or SVE 5 Kb) containing this viral gene into secondary cultures of rat kidney cells, as described above. Dishes were scored for foci after 3 to 4 weeks. We used recombinant plasmid pLP, derived from pBR322 and containing the authentic SV40 early genes, as a control. Comparisons between the recombinant DNAs containing super T antigen and plasmid pSt were made with 5 μg of DNA per plate. In this series of assays (Table 1), the transformation efficiency of the gene for super T antigen ranged from 20 to 30% of that of the gene for large T antigen. These results are in reasonable accord with those reported previously (26) and

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confirm that 115K super T antigen retains, to a fairly large extent, the transforming activity of SV40 large T antigen. On the other hand, the 115K super T antigen was shown to be markedly defective for initiating SV40 DNA synthesis (26).

In each experiment, representative foci induced by any one of the plasmid or cosmid preparations were picked, and the cells of each focus were expanded into a cell line. In some experiments, psT and pSVsT DNAs amplified in strain HB101 were linearized by complete digestion with SaI, which cuts these plasmids at the unique cleavage site (nucleotide 650) of pBR322, and were used for transformation. The several focus-derived cell lines were labeled with $^{32}$P and compared for the migration patterns of proteins immunoprecipitated with anti-SV40 tumor serum (Fig. 3).

The patterns (Fig. 3b, c, d, and e) show that all cell lines transformed by a recombinant DNA containing super T antigen gene expressed two classes of phosphoproteins immunoprecipitable with the anti-SV40 tumor serum. First, all of them contained a highly radioactive $^{32}$P-labeled antigen comigrating with the cellular p53 protein (18) which was, in fact, identified with the p53 protein by its immunoprecipitation with PAb421 monoclonal antibodies specific for large T antigen (Fig. 4). Thus, the migration patterns of immunoprecipitable protein regularly show a p53 band for every cell line transformed.

![Diagram](image)

**FIG. 2.** Comparative restriction maps of the genes encoding normal-size large T and 115K super T antigens. Only the relevant region is depicted. Line A, Region of the gene for large T antigen that is located between nucleotides 4675 and 2604; line B, the corresponding region of the gene for 115K super T antigen with an additional insertion of 666 bp as represented by the triangle; vertical arrows (↓), cleavage sites for restriction enzymes TaqI, HindII, and PstI. The probe used in this study is represented by the thin horizontal line at the top. The nucleotide numbering system used for SV40 DNA is as described in the text. The nucleotide numbers of the downstream 573-bp direct repeat unit are indicated by primes, and the nucleotide of the 93-bp inverted repeat separating both 373-bp direct repeat units are indicated by double primes (25, 26).

**TABLE 1.** Transformation of secondary rat kidney cell cultures by recombinant DNA containing the SV40 large T antigen gene (pLP) or the 115K super T antigen gene (SVE 5 Kb, psT, pSVsT)$^a$

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<tr>
<th>Recombinant DNA tested</th>
<th>Avg no. of foci per 10 plates</th>
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<tr>
<td>pLP$^b$</td>
<td>105</td>
</tr>
<tr>
<td>psT$^b$</td>
<td>19</td>
</tr>
<tr>
<td>pSVsT$^a$</td>
<td>25</td>
</tr>
<tr>
<td>pSVsT$^c$</td>
<td>25</td>
</tr>
<tr>
<td>SVE 5 Kb$^d$</td>
<td>32</td>
</tr>
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</table>

$^a$ Subconfluent monolayers of secondary rat kidney cells (2.5 x 10$^6$ cells seeded per 60-mm petri dish) were transfected with 5 μg of DNA per dish, and dense foci were counted 3 to 4 weeks later.

$^b$ Preparation of plasmid pLP, pSVsT, or psT used in this assay consisted essentially in a monomer population. To obtain this preparation, pLP, pSVsT, or psT was cleaved with BamHI, recircularized by ligation under dilute conditions, and amplified in E. coli HB101.

$^c$ This assay, the plasmid pSVsT was prepared as in footnote $b$ above and then linearized by SaI, which cuts this plasmid at the unique cleavage site (nucleotide 650) of pBR322.

$^d$ SVE 5 Kb DNA was propagated in E. coli 1106 and consisted in a mixture of monomer and multimer populations.
HOMOLOGOUS RECOMBINATION IN RAT CELLS

The cultures were grown in passage 3. The transformation of the cell lines was obtained by transfection with a plasmid containing the genes of interest. The same patterns were obtained when the cells were transfected with plasmids containing the genes of interest. The number of transformants per 10^6 cells was determined for each plasmid. The results were expressed as the number of transformants per 10^6 cells transfected with the plasmid of interest.
the N-terminal region of large T antigen for PAb405 and PAb414 and in the C-terminal region of large T antigen for PAb416 and PAb419 (15). In patterns of type iii, the possibility of interpreting the normal-size variant large T band to a degradation product seems unlikely since there are no immunoprecipitable proteins with higher molecular weights.

In the transformed cells with a migration pattern of type ii or iv, it is possible that the cells contain a gene encoding normal-size variant large T antigen. In addition, it is possible that the cell lines with the type ii migration pattern contain a gene encoding 115K super T antigen, and the cell lines with a type iv pattern are likely to contain genes encoding other variant T antigens such as truncated T antigen.

As judged by 32P-labeling experiments, a number of transformants (for instance pSV5-transformed lines 12 and 19) expressed only normal-size large T antigen in the absence of a detectable level of any other form of T antigen. This observation was confirmed by an experiment in which these latter transformants were labeled with [35S]methionine (Fig. 5). Moreover, it should be noted that the mobility patterns of

Among the second class of immunoprecipitable proteins, we found that the migration patterns can be divided into four types as follows: (i) presence of 115K super T antigen band (Fig. 3b, track 11); (ii) in addition to the 115K super T antigen band, presence of a band comigrating with SV40 wild-type (wt) large T antigen (90,000 Mr) (Fig. 3c, track 45); (iii) absence of the 115K super T antigen band but, instead, presence of a band comigrating with SV40 wt large T antigen (Fig. 3e, track 1); (iv) in addition to a band comigrating with wt large T antigen, presence of a band corresponding to ca. 75,000 Mr (Fig. 3b, track 14). This latter band probably corresponds to a truncated form of large T antigen. The expression of truncated T antigen by SV40-transformed cell lines is of relatively frequent occurrence (33). Moreover in the same pattern, the band comigrating with wt large T antigen is accompanied by a very close band corresponding to ca. 100,000 Mr (doublet).

The migration pattern of type i is characteristic of SV40-transformed cell lines expressing only the 115K super T antigen. In the transformed cell lines with a migration pattern of type iii, a new gene encoding a phosphoprotein with the size of large T antigen was probably created through recombinational event(s) undergone by the input gene encoding 115K super T antigen. The possibility that this phosphoprotein is a variant large T antigen is reinforced by the fact that the phosphoprotein is immunoprecipitated not only by the anti-SV40 tumor serum but also by any one of the monoclonal antibodies against large T antigen, of the series PAb405, PAb414, and PAb416 (Fig. 4). The binding sites of these monoclonal antibodies have been localized in the N-terminal region of large T antigen for PAb405 and PAb414 and in the C-terminal region of large T antigen for PAb416 and PAb419 (15). In patterns of type iii, the possibility of interpreting the normal-size variant large T band to a degradation product seems unlikely since there are no immunoprecipitable proteins with higher molecular weights.

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SVE 5 Kb-transformed lines 1 and 6 (Fig. 5) revealed the presence of a faint band of 115K super T antigen in addition to a major band of wt-size large T antigen.

There are four points worth noting here. (i) The cell lines derived by transfection with recombinant plasmid pLP containing the gene encoding SV40 large T antigen regularly showed the same familiar pattern with two bands, one corresponding to large T antigen and the other corresponding to the transformation-associated cellular p53 protein (18, 20, 22, 26); Fig. 3a).

(ii) The migration patterns of immunoprecipitable proteins from the various cell lines remained unchanged during the successive passages of all the cell lines tested (between passages 2 and 8; cf. Fig. 3b and Fig. 4). The expression of new variant T antigens should therefore result from the conversion of the gene encoding 115K super T antigen into a gene encoding a new variant T antigen(s), as would be expected if the input super T antigen gene underwent rearrangement mediated by recombination events.

(iii) A striking feature is the extraordinarily high frequency (called, for simplicity, recombinant frequency) of the formation of new variant T antigens in the transformed cell lines (Table 2). It should, however, be kept in mind that the methods used for revealing recombinant genes do not allow one to detect those recombinational events that involve the addition or deletion of only a few nucleotides or the formation of genes without retaining the transforming function. It is, therefore, impossible to assess the absolute recombination frequencies, although it seems obvious that the classical recombination frequencies are far lower than recombinant frequencies obtained in our system.

(iv) Another important point is that the new variant large T antigens observed in the transformed cell lines are predominantly those comigrating with wt large T antigen. This observation may be informative about the recombination events involved in the formation of the new variant T antigens.

To investigate whether these normal-size large T antigen variants might be identical to the wt SV40 large T antigen, the viral inserts contained in these transformants were subjected to restriction mapping by Southern blotting.

A close examination of the mapping data in Fig. 2 indicates that double digestion with TaqI and PstI of the gene for large T antigen (Fig. 2, line A) generated a 1,532-bp fragment extending from nucleotide 4675 (TaqI site) to nucleotide 3144 (PstI site), whereas super T antigen gene subjected to the same restriction treatment generated a 2,198-bp fragment, owing to the extra 666-bp sequence of super T antigen gene, as shown by the triangle (beneath line B) in Fig. 2.

When SV40 DNA, SVE 5 Kb DNA, and V 11 F 1 clone 1, subclone 7 cell DNA were doubly digested with TaqI and PstI and electrophoresed to serve as a standard, the 1,532-bp band was found in the lane of SV40 DNA and the 2,198-bp band was found in the lane of SVE 5 Kb DNA.

### Table 2. Expression of various forms of SV40 T antigens in recombinant DNA-transformed cell lines

<table>
<thead>
<tr>
<th>Transforming agent and resultant cell line</th>
<th>Form of T antigen expressed</th>
<th>115K super T</th>
<th>wt-size T</th>
<th>wt-size variant T</th>
<th>wt-size other variant T</th>
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<tr>
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<sup>a</sup> wt, Wild type.
<sup>c</sup> DNA of this transforming agent was propagated in E. coli 1106 and consisted of monomer and multimer populations.
<sup>d</sup> Recombinant frequency (percentage of total cell lines of the series expressing at least one recombinant T antigen), 27.
<sup>e</sup> Recombinant frequency of lines 1 through 19, 45; recombinant frequency of lines 21 through 36, 21.
<sup>f</sup> In these assays, the preparation of DNA consisted essentially of a monomer population, obtained as described in Table 1, footnote b.
<sup>h</sup> Recombinant frequency, 10.
<sup>i</sup> In this assay, pSV<sub>T</sub> DNA was prepared as described in Table 1, footnotes b and c.
<sup>j</sup> Recombinant frequency, 50.

<sup>k</sup> Each cell line is designated by the number of the focus from which it is derived.
FIG. 6. Southern blots of genomic DNA from transformed cell lines. All DNAs were doubly digested either with (a) and (c) TaqI and PstI or (b) with TaqI and HindII, electrophoresed on 1% agarose gel, and blotted to the probe described in the text and illustrated in Fig. 2. The penultimate lane (SV40 DNA) of each gel shows a digested mixture of SV40 DNA and BMK cell DNA. The lane labeled SVE 5 Kb cosmid contains a digested mixture of SVE 5 Kb cosmid DNA and BMK cell DNA. In each gel, lane M contains restriction fragments of SV40 DNA as size markers.

band was found in the lane of SVE 5 Kb cosmid DNA and V 11 F 1 clone 1, subclone 7 cell DNA (Fig. 6).

Similarly, the mapping data in Fig. 2 show that double digestion of large T or super T antigen gene with TaqI and HindII generated a 1,005-bp fragment extending between nucleotides 4675 and 3671. The occurrence of an extra HindII site at nucleotide 3671' in super T antigen gene was reflected by the presence in the Southern blot pattern of an extra 666-bp band corresponding to the fragment extending between nucleotides 3671 and 3671'.

The Southern blot patterns predicted for the DNAs of SV40, SVE 5 Kb cosmid, and V 11 F 1 clone 1, subclone 7 are in good accord with the experimental results (Fig. 6).

In addition, as judged by the Southern blotting patterns (Fig. 6a, b, and c) the normal-size variants of large T antigen, present either in pSVsT-transformed cell lines 12 and 19 or in SVE 5 Kb-transformed lines 1 and 6, are indistinguishable from wt large T antigen. Note that the super T antigen gene present in SVE 5 Kb-transformed line 6 is also detectable in the Southern blot pattern in Fig. 6c.

DISCUSSION

In this study we analyzed the migration patterns of immunoprecipitable proteins from transformed cells obtained after transfection of secondary rat cell cultures with hybrid plasmid or cosmid DNAs containing a gene encoding SV40 115K super T antigen. The results (Fig. 3 and Table 2) revealed that in a very large proportion of transformants (up to 50%) the gene for 115K super T antigen is converted into genes encoding new variant SV40 T antigens, the most
predominant class of variants being of a size similar or identical to that of wt SV40 large T antigen.

Comparable results were obtained with plasmid or cosmid DNA preparations consisting of either a monomer population or a mixture of monomer and multimer populations (Table 2). The linearized form of pSVST DNA, however, seems to be relatively less subject to rearrangements. The recombinant viral gene is likely to be integrated into the host cell genome since the migration patterns of immunoprecipitable proteins remained unchanged during the successive passages tested. In fact, we have observed that, in SVE 5 Kb-transformed cell line 1 at passage 6, all the viral sequences are linked to high-molecular-weight chromosomal DNA as judged by restriction mapping and hybridization studies (data not shown).

Based on the results obtained by others concerning the recombination between transferred DNA sequences (1, 6, 14, 29, 30, 37, 38, 41, 42, 45, 46, 48), it appears likely that the input plasmid or cosmid DNAs are first subjected to recombination events yielding the variant T antigen genes before these recombinant genes become integrated into high-molecular-weight DNA, although we cannot rule out the possibili-

ty that the recombination events actually follow the integration of viral sequences.

We have already emphasized that the results shown in Table 2 cannot be expressed in terms of absolute recombination frequencies. Moreover, the particular methods that we used to obtain transformants and to reveal recombinant genes limited this study to a particular class of recombinants.

It is unlikely that the very frequent conversion of super T antigen genes into genes encoding normal-size variant T antigens occurs in a chance manner. Several kinds of reciprocal recombination events would account for such conversion (Fig. 7). First, an intermolecular, unequal crossing over would generate two types of plasmid or cosmid DNA, one of which would have lost the extra sequence of DNA contained in 115K super T antigen gene (from nucleotides 4776' to 3544' [Fig. 2]) and the other of which would have gained this extra sequence (Fig. 7a). Alternatively, the wt large T antigen gene could also be regenerated by an intramolecular recombination of the input plasmid or cosmid DNA involving the pairing of the homologous (5' or 3') termini of both repeating units contained in the super T antigen gene. The resulting exchange would provoke the excision of the aforementioned extra DNA sequence. The deleted material might exist as a small circle (Fig. 7b).

If our hypothesis is correct, it implies that the normal-size recombinant large T antigens are identical with wt large T antigen. In fact, normal-size recombinant large T antigens are indistinguishable from the wt large T antigen, as judged by (i) their electrophoretic mobility, (ii) their immunological reactions with a series of monoclonal antibodies, and (iii) restriction mapping of the total genomic DNA of the transformants by Southern blotting.

Our results and interpretation closely resemble those obtained with a different system (17, 44). These earlier findings showed that the heterogeneous adenovirus-SV40 hybrid Ad2++ HEY consists of an adenovirus helper virion, a nonhybrid SV40 virion, and three defective hybrid virions (HEY 0.4, HEY 1.4, and HEY 2.4) whose genomes differ in SV40 DNA (0.45, 1.43, and 2.39 SV40 genomes, respectively). In these hybrids that contain more than one complete SV40 genome (HEY 1.4 and HEY 2.4), the excess SV40 DNA was shown to be organized as a tandem repetition. Data reported by these authors strongly suggest that the various hybrid genomes within each population are intercon-vertible by recombination events, which inserted or excised an SV40 genome.

It is worth stressing that the rat cells are nonpermissive or semipermissive for SV40 (40) and that 115K super T antigen lacks the ability to support SV40 DNA replication (26). Therefore, the recombination events studied here are likely to occur between or within nonreplating molecules such as molecules of the input plasmid or cosmid DNA.

The frequent expression of normal-size large T antigen variants by foci of transformed cells, after transfection with the cloned gene for 115K super T antigen, indicates that 115K super T antigen has no selective superiority over these large T antigen variants in inducing cell transformation. This fact indicates some correspondence with the fact reported earlier (26) and confirmed here (Table 1) that 115K super T antigen has a moderately reduced ability to initiate cell transformation, as compared with large T antigen. In contrast, it appears that, once established, the cell lines transformed by 115K super T antigen are stable. In that respect, the phenotype of V 11 F 1 clone 1, subclone 7 cell line has been remarkably stable in continuous passage since its isolation in 1979, including its permanent expression of 115K
super T antigen (26); this observation provides evidence that the repeated sequence can exist in a stable form.

Concerning the normal-size large T antigen(s) expressed in the transformed cells listed in Table 2, the question remains open as to whether these normal-size large T antigens possess a replicative ability. Such a possibility can only be tested by cloning these genes.

It is worth noting that the mechanisms involved in the interconversion between the genes for the 115K super T antigen and for the normal-size large T antigen are reminiscent of the mode of sequence rearrangement leading to continual expansion and contraction of the repeated sequences observed during evolution of eucaryotes (11).

ACKNOWLEDGMENTS

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LITERATURE CITED


