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Cellular Mutation Mediates T-Antigen-Positive Revertant Cells Resistant to Simian Virus 40 Transformation but Not to Retransformation by Polyomavirus and Adenovirus Type 2

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T-antigen-positive transformation revertant cell lines were isolated from fully simian virus 40 (SV40)-transformed Fisher rat embryo fibroblast cells (REF 52 cells) by methionine starvation. Reversion of the transformed cells (SV-52 cells) was caused by a mutation within the cellular genome. To demonstrate this, we isolated SV40 DNA from the host genome, inserted it into plasmid pSPT18 DNA, cloned it in *Escherichia coli*, and microinjected it into the nuclei of the REF 52 cells. Fully transformed cells were obtained with the same efficiency (20 to 25%) as after microinjection of wild-type SV40 DNA I. Furthermore, the revertant cells were resistant to retransformation by SV40. Following microinjection of wild-type SV40 DNA I, 42 independent cell lines were isolated. Cells of all analyzed lines acquired additional SV40 DNA copies, but changes in the cell morphology or growth characteristic were not demonstrable. However, the revertants were retransformable with a high efficiency after polyomavirus and adenovirus type 2 infections or microinjection. Also, fusion of the revertant cells with the grandparental REF 52 cells led to restoration of the transformed state.

The transformation potential of simian virus 40 (SV40) has been under extensive investigation during the last three decades. Experiments with early viral mutants (*ts*A, *dl*59/54) revealed that synthesis of the large T antigen is required and sufficient to induce tumor formation and oncogenic transformation of tissue culture cells (26). SV40 DNA fragment injection experiments have further shown that the first T-antigen exon is dispensible for maximal cell transformation (8).

In the meantime, many details about the T antigen have become known. This 92-kilodalton phosphoprotein is a pleiotropic effector with numerous biochemical and biological functions. To name only a few, the SV40 T antigen is a DNA-binding protein with an inherent ATPase and an associated protein kinase activity, it stimulates cellular and viral DNA replication, it induces enhanced production of cellular proteins, such as the p53 tumor antigen, and it causes alterations in cell morphology and growth characteristics (6, 16, 19, 20, 22, 26).

So far it is not known which T-antigen function(s) is crucial for oncogenicity. However, it is evident from different types of experiments that T-antigen synthesis per se is not sufficient to cause cell transformation (10, 11, 13, 23, 27; see Discussion). Cell transformation requires the direct or indirect interplay of the T antigen with cellular molecules (target molecules). The nature of these target molecules is still a matter of speculation.

Our approach to the analysis of this cellular T-antigen target system in further detail involves the characterization of transformation revertants. In this study, we describe two flat, large- and small-T-antigen-positive cell lines which were isolated by methionine starvation from SV40-transformed Fisher rat embryo fibroblast cells (REF 52 cells). Reversion of the fully transformed cells to cells with the growth characteristics of the grandparenteral REF 52 cells was caused by a stable alteration of the cellular genome which caused the cells to be resistant to SV40 retransformation but not to retransformation by polyomavirus or adenovirus type 2.

MATERIALS AND METHODS

Cells. For all experiments, REF 52 cells were used. Unless indicated otherwise, cells were grown in Dulbecco medium (DMEM) supplemented with 5% (vol/vol) fetal calf serum (FCS) (GIBCO Laboratories). For miroinjection experiments, cells were grown on glass slides subdivided into small numbered squares. This procedure allows identification and isolation of the recipient cells at any time after DNA transfer (3). SV40-transformed REF 52 cells were obtained after microinjection of SV40 DNA or SV40 DNA fragments as described in detail elsewhere (8). For anchorage-independent growth, cells were seeded into 0.33% Sea-Plaque agarose (FMC Corp., Marine Colloids Div.). The agarose concentration for the bottom layer was 0.5%.

For cell fusion, confluent cultures were washed twice with serum-free medium, and polyethylene glycol 1000 (Koch-Light Laboratories Ltd.) was added for 45 s to the cells (45% [wt/vol] PEG 1000 in DMEM without serum). After aspiration of the PEG 1000 solution, the cells were washed three times with DMEM containing 15% (vol/vol) dimethyl sulfoxide and rewashed several times with DMEM supplemented with 5% FCS. Cells were then incubated under standard conditions and washed with DMEM with serum 2 to 5 h later (4).

Immunoprecipitation of T antigen. After being labeled with [^{35}S]methionine (0.1 mCi/ml) for 2 h, cells were washed with phosphate-buffered saline and lysed by the addition of 1% (vol/vol) Nonidet P-40 in 20 mM Tris hydrochloride (pH 8)–0.15 M NaCl–50 μg of phenylmethylsulfonyl fluoride per ml–20 μg of L-1-tosylamide-2-phenylmethyl chloromethyl ketone per ml for 15 min at 4°C. The lysates were transferred into Eppendorf tubes, diluted with NET buffer (0.15 M NaCl, 5 mM EDTA, 50 mM Tris hydrochloride [pH 7.4], 0.05% Nonidet P-40, 0.02% [wt/vol] NaN_3) with 0.2%

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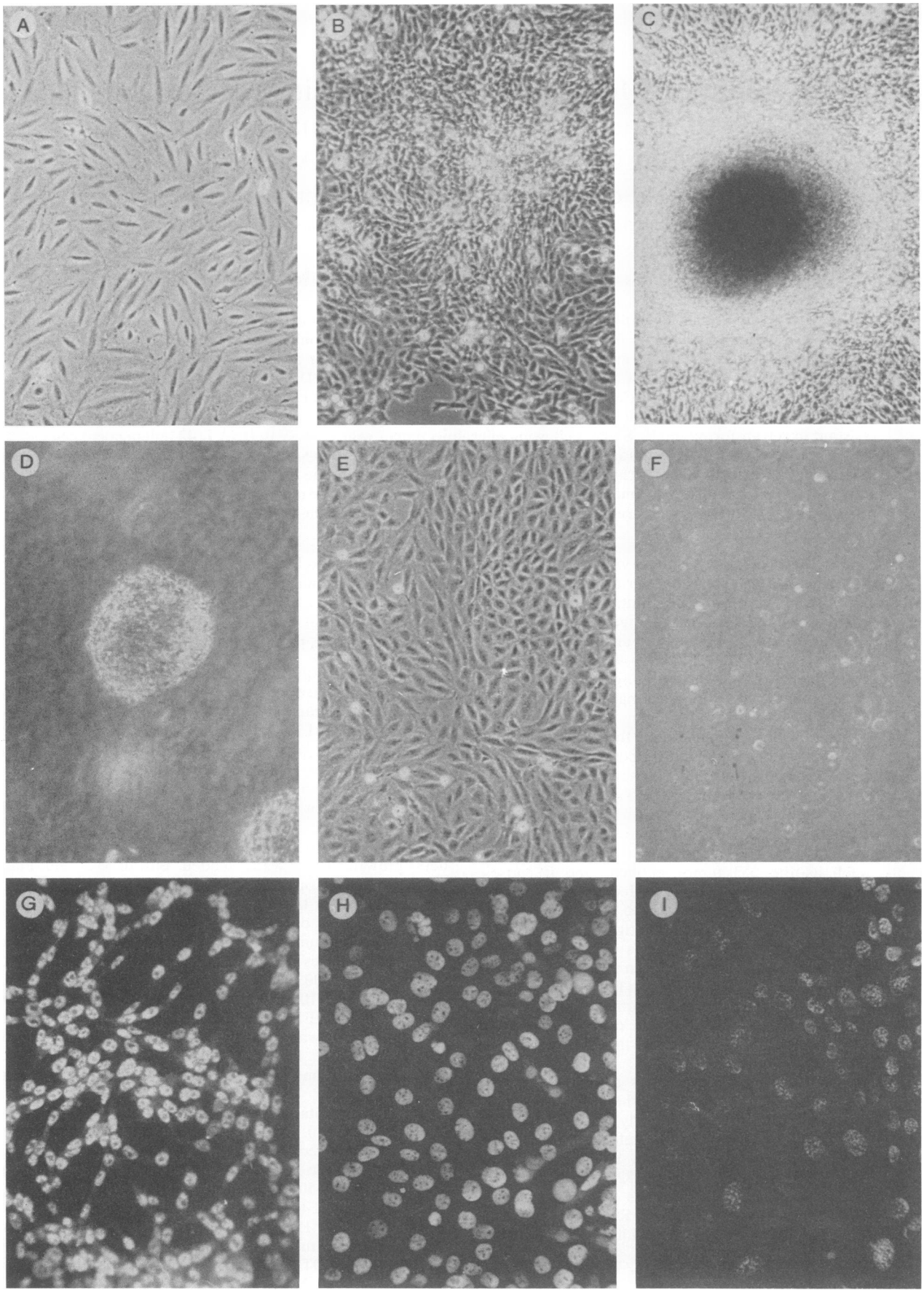
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(wt/vol) bovine serum albumin to a final volume of 600 μl, treated with 30 μl of protein A fixed to cell walls of inactivated *Staphylococcus aureus* suspension (IgG sorb; The Enzyme Center Inc.) for 15 min at 4°C, and then centrifuged for 2 min in an Eppendorf centrifuge. For immunoprecipitation, the supernatants were treated with 5 μl of hamster anti-T serum by incubation at 4°C overnight. The antigen-antibody complexes were removed from the solution by treatment with 15 μl of 10% (wt/vol) IgG sorb for 15 min at 4°C and centrifugation for 2 min. Pellets were then washed four times with NET buffer without albumin at 4°C and suspended in 20 μl of gel sample buffer for 30 min at 4°C. Samples were analyzed on 7 to 15% discontinuous polyacrylamide gels. After fluorography, dried gels were exposed for 1 to 2 days to Kodak XAR-5 films at −70°C.

Isolation of cellular DNA, blot analysis, and molecular cloning. High-molecular-weight DNA was extracted from the culture cells by standard procedures (7). Genomic DNA (5 to 10 μg) was digested with different restriction endonucleases, and the products were submitted to electrophoresis on horizontal 1% agarose gels. DNA fragments were transferred to nitrocellulose filter sheets and hybridized with nick-translated [^{32}P]DNA probes (specific activity, 1×10^8 to 2×10^8 cpm/μg) as described previously (24).

For preparation of the SV40 DNA insert, isolated cellular DNA (100 μg) from Rev2 cells (see below) was digested with *Eco*RI and subjected to preparative agarose gel electrophoresis (1%). As a size marker, lambda DNA digested with *Eco*RI was used. The SV40 DNA-containing region (identified with a control gel and a DNA blot) was cut out and eluted from the gel as described previously (8). The isolated DNA was inserted into the *Eco*RI site of the pSPT18 plasmid vector (Pharmacia, Inc.) and propagated in *Escherichia coli* BHB2603. Filter hybridization was used for identification of the SV40 DNA-containing clones (17).

RESULTS

Isolation and characterization of T-antigen-positive transformation revertant cell lines. Flat T-antigen-positive revertant cell lines were isolated from fully SV40-transformed REF 52 cells. For selection of the revertants, confluent cultures of SV-52 cells which had been recloned three times in soft agar were fed with serum- and methionine-free DMEM for 5 days under standard culture conditions. During this starvation period, the majority of the transformed cells were dying, and only small islands of flat cells survived this treatment. Cells of these islands were isolated under a phase-contrast microscope by aspiration into glass needles (diameter at the tip, about 20 μm), transferred into microwell dishes, and further cultivated in DMEM supplemented with 5% FCS.

In this way, 15 independent flat cell lines were obtained; two cell lines (Rev1 and Rev2) had T-antigen-positive cells, as assayed by indirect immunofluorescence staining (Fig. 1H). A strong intranuclear fluorescence was also obtained with antisera specific for the first 8 amino acid residues of the N terminus of the T antigen or for the last 11 amino acid residues of the C-terminal region of the T antigen (antisera were kindly provided by G. Walter).

To determine the size of the T antigen synthesized in the revertants, we incubated Rev1 and Rev2 cells in DMEM with [^{35}S]methionine for 2 h, immunoprecipitated protein extracts with hamster anti-T serum, and subjected the extracts to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Rev2 cells synthesized large T antigen and small t antigen of similar sizes and in comparable quantities as those synthesized by the parental SV-52 cells (Fig. 2). Rev1 cells contained a reduced amount of T antigen. Elevated concentrations of the cellular p53 tumor antigen were also demonstrable by immunofluorescence staining (Fig. 1I) and by gel electrophoresis (Fig. 2).

The morphology of the revertants is shown in Fig. 1E.

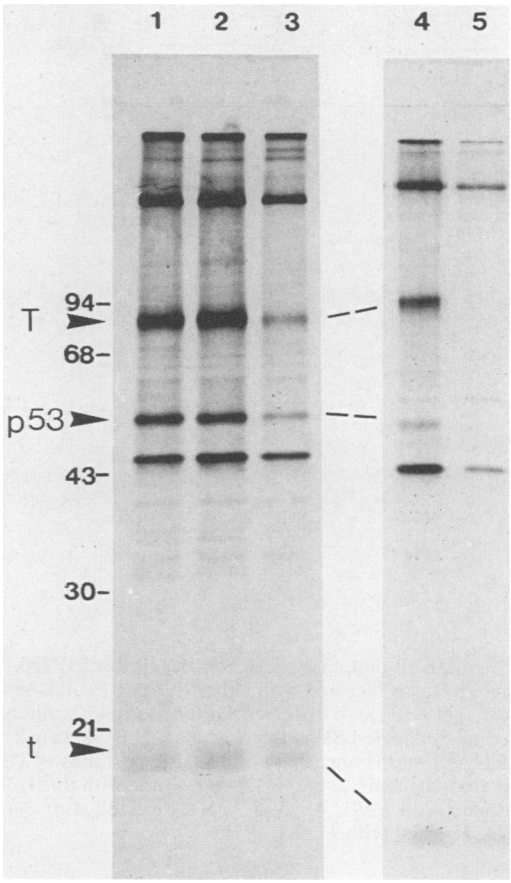

FIG. 2. Fluorogram of a sodium dodecyl sulfate-polyacrylamide gel (7 to 15%) of proteins immunoprecipitated with anti-T serum from the following cells: lane 1, SV-52; lane 2, Rev2; lane 3, Rev1; lane 4, REF 52 transformed by the SV40 *Hpa*II-*Bam*HI fragment isolated from the Rev2 cells; and lane 5, REF 52. Cells cultivated on plastic dishes (about 10^6) were labeled with [^{35}S]methionine (0.1 mCi/ml; specific activity, 1,470 Ci/mmol; Amersham Corp.) for 2 h at 37°C. Protein extracts were immunoprecipitated with a polyclonal anti-T-serum from tumor-bearing hamsters. The positions of the SV40 large (T) and small (t) T antigens and the p53 protein are indicated. The following molecular mass markers were used: phosphorylase *b* (94,000 daltons), bovine serum albumin (68,000 daltons), ovalbumin (43,000 daltons), carbonic anhydrase (30,000 daltons), and trypsin inhibitor (21,000 daltons). A similar protein pattern was obtained with a monoclonal anti-p53 serum (kindly provided by E. Gurney) for immunoprecipitation.

FIG. 1. Morphology of SV40-transformed and revertant cells (phase-contrast photographs). (A) Confluent REF 52 cells; (B) semiconfluent SV-52 cells; (C) confluent SV-52 cells; (D) SV-52 cells 2 weeks after plating on soft agar; (E) confluent Rev2 cells; (F) Rev2 cells 3 weeks after plating on soft agar; (G) T-antigen-positive SV-52 cells; (H) T-antigen-positive Rev2 cells; (I) Rev2 cells stained with anti-p53 serum.

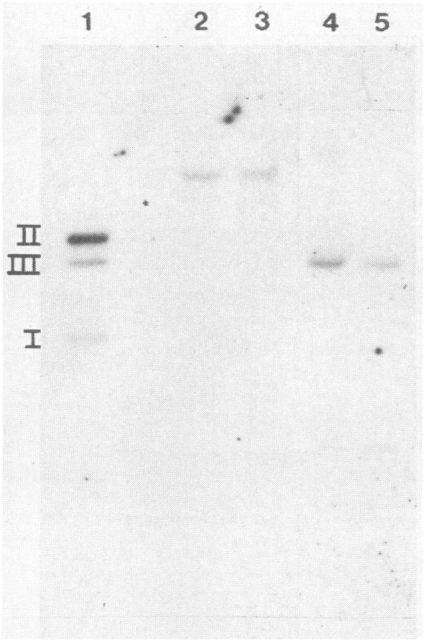

FIG. 3. Southern blot analysis of SV-52 and Rev2 DNAs. Cellular DNA (10 μg) was digested with either *Bgl*II or *Eco*RI, separated on agarose gels (1%), transferred to nitrocellulose filters, and hybridized to ^{32}P-labeled SV40 DNA. Lanes: 1, SV40 marker DNA (5 pg of DNAs I and II and 1 pg of DNA III; positions of DNAs I, II, and III are indicated); 2, SV-52 DNA digested with *Bgl*II; 3, Rev2 DNA digested with *Bgl*II; 4, SV-52 DNA digested with *Eco*RI; 5, Rev2 DNA digested with *Eco*RI.

Rev1 and Rev2 cells were flat and larger than the parental SV-52 cells (Fig. 1B) and formed only monolayers on the culture dish with 5 and 10% FCS concentrations (Fig. 1E). Focus formation was not demonstrable (data not shown). As a further criterion for transformed cells, the growth characteristics of the revertants in soft agar were tested. Rev1 and Rev2 cells were plated on soft agar at different input concentrations (10^3 to 10^5 cells per 60-mm dish) and with 5% FCS and were monitored for 4 to 5 weeks. Under these conditions, the Rev1 and Rev2 cells remained as single cells, and colony formation was not demonstrable. In contrast, 80% of the SV-52 cells formed large colonies (Fig. 1D). We also tested the anchorage-independent growth of the Rev1 and Rev2 cells with 10 and 20% (final) FCS concentrations and found that a small number of the cells (0.1 to 0.3%) formed minicolonies with a maximum of 16 cells. Following isolation, cells of these minicolonies exhibited the same growth characteristics as the Rev1 or Rev2 cells. The phenotype of the Rev1 and Rev2 cells described above remained stable during continuous cultivation following isolation for approximately 1.5 years. To test the state of the SV40 DNA in the revertants and in the transformants, we extracted cellular DNA from the Rev2 and SV-52 cells, digested it with *Bgl*II endonuclease (which does not cut SV40 DNA), and subjected it to DNA blot analysis. One SV40 insertion was demonstrable in both the transformants and the revertants (Fig. 3).

Transformation capacity of Rev2 cell SV40 DNA. Going one step further, we wondered whether the reversion of the fully transformed SV-52 cells to cells with the growth characteristics of the grandparental REF 52 cells (Fig. 1A) was caused by an alteration of the host genome or by a mutation within the early SV40-coding region. Since SV40 virus rescue was not demonstrable after fusion of the revertant cells with either TC7 or Cos7 cells, the genomic DNA of the Rev2 cells was cleaved with *Eco*RI endonuclease, which cuts SV40 DNA only within the late coding region. This treatment generated an SV40 DNA-containing band of about 5.2 kilobases (Fig. 3) which, after isolation, was inserted into the *Eco*RI site of plasmid pSPT18 DNA and propagated in *E. coli*. The 5.2-kilobase fragment contained the whole early region of the SV40 DNA (promotor-enhancer and T-antigen-coding part), and this region was isolated from the purified plasmid DNA by *Hpa*II-*Bam*HI endonuclease treatment and agarose gel electrophoresis.

The transformation capacity of the *Hpa*II-*Bam*HI DNA fragment was tested following intranuclear injection into the REF 52 cells. About 20% of the injected cells grew into permanently T-antigen-positive cell lines which in all respects analyzed (cell morphology, serum requirement, focus formation, and growth in soft agar) corresponded to wild-type SV40 transformants. T-antigen-positive cells with the morphology and growth characteristics of the Rev2 cells were not obtained. These experiments showed that Rev2 SV40 DNA still contained the full transformation capacity of wild-type SV40 DNA I.

Specificity of resistance of Rev2 cells to retransformation by SV40 but not by polyomavirus and adenovirus type 2. The results discussed above support the assumption that the reversion was caused by an alteration of the cellular genome, conferring on the Rev1 and Rev2 cells insensitivity to the oncogenic transformation potential of the SV40 T antigen. Therefore, we must assume that the revertants remained resistant to retransformation upon SV40 DNA injection. To clarify this assumption, we microinjected 400 Rev2 cells grown on glass slides subdivided into small numbered squares (3) with SV40 DNA I and pSV-2 neo DNA (which encodes resistance to the drug G418). Two days after injection, G418 (0.5 mg/ml) was added to the culture medium. Three weeks later, 42 independent cell clones (Rev-SV-Neo 1 to 42) were isolated and further propagated in the presence of G418. The morphology of the cells (Fig. 4A) was indistinguishable from that of the Rev2 cells (Fig. 1E). Following plating on soft agar, cells of all 42 lines did not grow with 5% FCS. With high FCS concentrations (10 or 20%), only cells of lines Rev-SV-Neo 31 to 42 exhibited as low a division rate as the Rev2 cells. To determine whether the Rev-SV-Neo cells acquired new SV40 DNA insertions, we isolated cellular DNA from three arbitrarily chosen clones (Rev-SV-Neo 1, 11, and 41), cleaved it with *Sst*I endonuclease (which does not cleave SV40), and subjected it to DNA blot analysis. These experiments showed that the Rev-SV-Neo cells acquired one or more new SV40 DNA insertions, as shown for Rev-SV-Neo 11 cellular DNA in Fig. 5.

Since Rev1 and Rev2 cells were found to be resistant against retransformation by exogenous SV40 DNA, we wondered whether polyomavirus could retransform these cells. To test this possibility, we microinjected 50 Rev2 cell nuclei with 20 to 40 polyomavirus DNA I molecules each. About 3 weeks after the DNA transfer, 18 morphologically transformed cell clones were isolated and further cultivated. Cells of these clones were, in all respects analyzed, fully transformed. The morphology of these cells is shown in Fig. 4C, and the anchorage-independent growth is summarized in Table 1. Furthermore, the Rev1 and Rev2 cells could be efficiently retransformed by adenovirus type 2. About 3 weeks after infection of Rev2 cells with 5 to 10 PFU of adenovirus type 2 per cell, large numbers of transformed

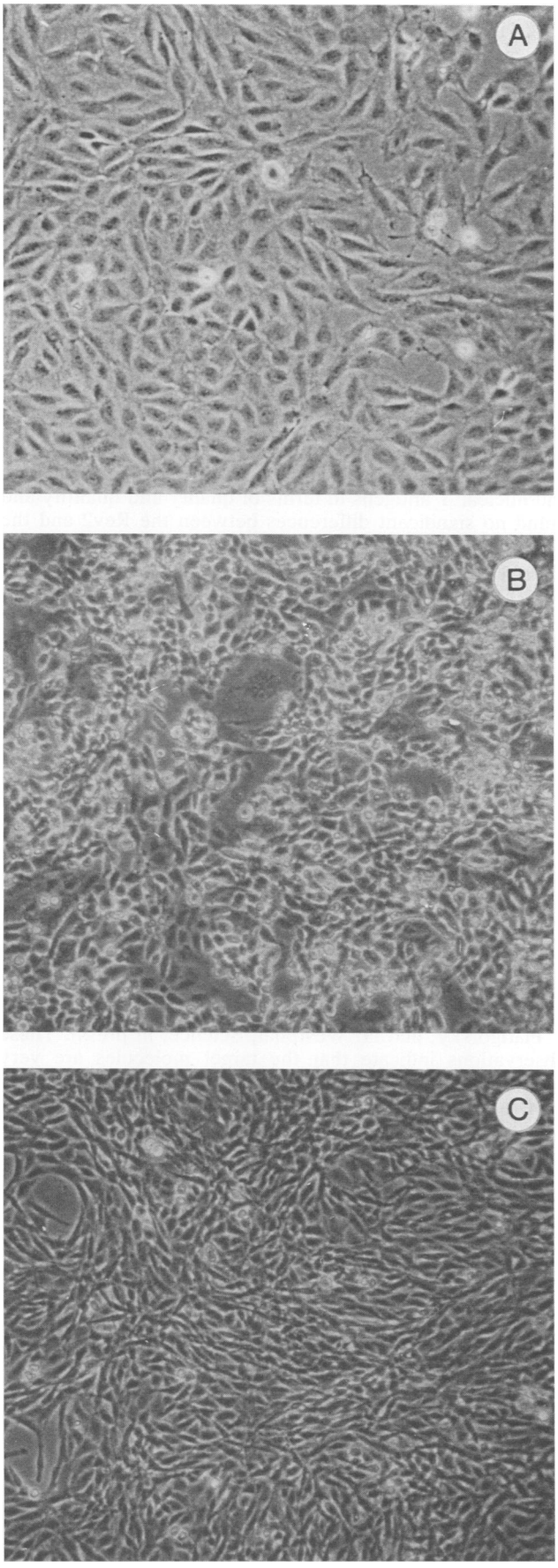

FIG. 4. (A) Confluent culture of Rev-SV-Neo 11 cells. (B) Rev2 cells transformed by adenovirus type 2. (C) Polyomavirus-transformed Rev2 cells.

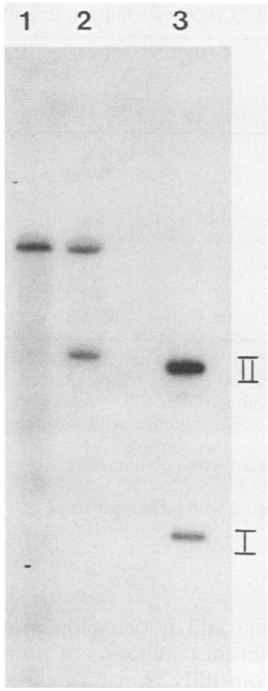

FIG. 5. Southern blot of *Sst*I-digested DNA after hybridization with nick-translated SV40 DNA. Lanes: 1, Rev2 cell DNA (10 μg); 2, Rev-SV-Neo 11 cell DNA (10 μg); 3, SV40 marker DNA (5 pg of DNAs I and II; positions of DNAs I and II are indicated).

colonies (more than 100 colonies per 60-mm plate) appeared on the culture dish. Three of them were isolated and further analyzed. Following plating on soft agar, cells of all three colonies formed with 70 to 80% efficiency large colonies with 5 and 10% FCS concentrations (Table 1). The morphology of the adenovirus type 2-transformed Rev2 cells is shown in Fig. 4B.

Finally, we wondered whether the T antigen synthesized in the Rev2 cells still carried its full transformation potential. For this purpose, Rev2 cells were mixed with REF 52 cells at a 1:1 ratio and plated on 60-mm dishes. Two days later, cells were fused by the addition of PEG 1000 as described in Material and Methods. After 48 h, cells were trypsinized, and aliquots were transferred to soft agar. Depending on the fusion efficiency, about 1 to 5% of the cells grew into large colonies with 5% FCS. The morphology of these cells was indistinguishable from the morphology of the SV-52 cells. After replating on soft agar, colonies formed with high efficiency (70 to 80%). Control experiments included fusion (via PEG 1000) of Rev2 cells with Rev2 cells and of REF 52 cells with REF 52 cells. Transformed cells were not obtained (Table 2).

DISCUSSION

It is evident that SV40 gene expression is required and sufficient for tumor formation in animals (e.g., hamsters and mice) and for oncogenic transformation of tissue culture cells (20a, 22, 26). Cotransfer of other oncogenes is not required. However, if selective pressure is omitted (e.g.,

TABLE 1. Colony formation in soft agar[a]

Cells	% of input cells forming colonies with:	
	5% FCS	10% FCS
REF 52	0	0
SV-52	70–80	70–80
Rev1	0	0.1–0.3[b]
Rev2	0	0.1–0.3[b]
Rev-SV-Neo 1 to 30	0	0
Rev-SV-Neo 31 to 42	0	0.1–0.3[b]
Rev2-PV[c]	80	NT[d]
Rev2-Ad2[e]	80–90	80–90

[a] Cells were plated (10^4 cells per 60-mm dish) as described in Materials and Methods with the FCS concentrations indicated. Cell clones were counted 3 weeks after transfer to soft agar.
[b] A small number of small colonies with a maximum of 16 cells were observed.
[c] Rev2 cells microinjected with polyomavirus.
[d] NT, Not tested.
[e] Rev2 cells micorinjected with adenovirus type 2.

growth on soft agar) and if cell clones are selected by a second marker, different categories of transformants can be isolated, ranging from fully transformed cells to T-antigen-positive cells with the morphology and growth characteristics of the normal parental cells. This diversity of phenotypical responses does not correlate with a low or high rate of T-antigen synthesis and cannot be explained by a specific integration pattern of the viral DNA into the host genome. Most likely, this phenomenon reflects a variation in the accessibility of the cellular target molecules for the oncogenic potential of the SV40 T antigen. Furthermore, cells which are once fully transformed can revert to normal. Vogel et al. (29, 30) described flat T-antigen-positive revertants which released transforming virus after fusion with permissive monkey cells. Since the revertant cells used in their experiments contained more than one SV40 copy and since rescue can lead to DNA recombination, a mutation within the early SV40-coding region as the reason for cell reversion cannot entirely be excluded.

In this investigation, we demonstrated that the reversion from fully transformed SV-52 cells to flat T-antigen-positive cells with the morphology and growth behavior of the grandparental REF 52 cells was caused by a stable genetic alteration within the host genome and not by a mutation within the early SV40-coding region. Evidence for this includes the following observations. (i) The SV40 DNA isolated from the Rev2 cells (*Hpa*II-*Bam*HI DNA fragment) transformed the REF 52 cells with the same efficiency as did the corresponding wild-type SV40 DNA fragment after intranuclear microinjection. (ii) The revertants synthesized large and small T antigens of similar quality and in comparable quantities as did the parental SV-52 cells. Furthermore, cell hybrids obtained after fusion of the Rev2 cells with the REF 52 cells were fully transformed (Table 2). This observation excludes the possibility that transformation of the REF 52 cells by SV40 was caused by a recessive mutation of a cellular gene(s) (9). (iii) Last but not least, the revertants were resistant to retransformation by SV40. Following coinjection of SV40 DNA I and pSV2 neo DNA, 42 independent cell lines were isolated by G418 selection. Cells of these lines acquired new SV40 DNA insertions but maintained the morphology and growth characteristics of the revertant cells. Importantly, this resistance was specific for SV40; the revertants could be retransformed with very high efficiency by either polyomavirus or adenovirus type 2.

These observations led us to question which cellular molecules mediated the resistance of Rev1 and Rev2 cells to the transformation potential of the SV40 T antigen. So far, very little is known about the nature and intracellular location of these target molecules. If cell transformation requires a direct interplay with the T antigen, the target molecules could be nuclear, cytoplasmic, or a plasma membrane component (1). Experiments with transport-negative mutants did not clarify the question as to whether intranuclear accumulation of the T antigen is essential for manifestation of the transformed state. Cells transformed by these mutants still may contain small amounts of nuclear T antigen not detectable by immunofluorescence staining but sufficient for cell transformation (12, 14, 15, 28, 31). Microinjection experiments have shown that standard immunofluorescence staining is a very insensitive technique and requires a minimum of 20,000 to 40,000 T-antigen molecules for a clear intranuclear fluorescence signal (5). We analyzed the plasma membrane and nuclear T antigens in terms of quality and quantity and found no significant differences between the Rev2 and the SV-52 cells (plasma membrane data are not shown). A well-characterized nuclear protein interacting directly with the SV40 T antigen is the cellular p53 tumor antigen (2, 16, 21). The fact that the revertant cells contained the p53 protein in concentrations similar to those in the parental SV-52 cells indicates that its enhanced expression and complex formation with the T antigen were not sufficient for the maintenance of the transformed state, in accordance with results reported elsewhere (10).

SV40 has the capability to transform different cell types of a large number of different species, including insects, amphibians, rodents, ungulates, and primates (26; unpublished observations). Experiments with transgenic mice have further demonstrated that cells of almost any organ are accessible to SV40 transformation (20a). Also, cells which are excluded from tumorigenicity in nature, such as the lens cells, became malignant transformed cells following microinjection of an alpha lens crystallin-SV40 T antigen hybrid DNA molecule (alpha lens crystallin-promoter T-antigen-coding region) into fertilized mouse eggs (K. A. Mahon, A. B. Chepelinsky, J. S. Khillan, P. A. Overbeek, J. Piatigorsky, and H. Westphal, Science, in press). These observations indicate that the target molecules are very common among different species.

So far it is not known whether the SV40 target systems are constitutive or adaptative molecules. Temperature shift experiments with cells transformed by temperature-sensitive mutants (*ts*A) revealed that the phenotype and growth characteristics of the cells can change from transformed to normal within hours (18, 20, 25). Following microinjection of SV40 DNA, the recipient cells acquired the morphology of

TABLE 2. Morphology and growth characteristics of hybrid cells[a]

Hybrid cells	Morphology	% of input cells growing in soft agar with 5% FCS
REF 52-REF 52	Normal	0
Rev2-Rev2	Normal	0
Rev2-REF 52	Transformed	1–5

[a] For growth in soft agar, cells were transferred to soft agar 2 days after fusion with PEG 1000 and were monitored for 4 weeks.

transformed cells within 2 to 3 days, and about 25 to 30% of them grew into permanently T-antigen-positive cell lines. However, changes in cell morphology did not correlate with anchorage-independent growth. Approximately 4 to 6 weeks after the isolation of the T-antigen-positive clones, the majority of the cells exhibited the morphology of transformed cells, but only a small proportion of them (less than 1%) grew in soft agar. This result indicates that changes in cell morphology are an acute T-antigen function, while colony formation in soft agar, the major in vitro criterion for tumor cells, occurs only after a latency period of several weeks and affects only a small subpopulation of the T-antigen-positive cells.

In this regard, the revertant cells are not entirely reconverted to a normal state, because both changes in cell morphology and anchorage-independent growth were reinducible in an acute fashion. Following fusion of the Rev2 cells with the normal REF 52 cells, colony formation in soft agar was demonstrable with high efficiency and without a lag period (Table 2). This result indicates that the REF 52 cells provided the hybrid cells with a factor which is essential for cell transformation and is not present in an active form in the revertant cells.

Furthermore, revertant cells can be retransformed with high efficiency after either polyomavirus or adenovirus type 2 infection. This observation supports the assumption that the *onc* proteins of these viruses follow pathways for maximal cell transformation that are different from those of the SV40 T antigen or that they use the same target molecules with different specificities.

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