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Transformation of Precrisis Human Cells by the Simian Virus 40 Cytoplasmic-Localization Mutant pSVCT3 Is Accompanied by Nuclear T Antigen

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pSVCT3 is a cytoplasmic-localization mutant of simian virus 40 (SV40) isolated from the SV40 adenovirus 7 hybrid virus (PARA) and cloned into plasmid PBR. The large T antigen of pSVCT3 accumulates in the cytoplasm of infected monkey cells instead of being transported to the nucleus. The sole change in CT3 large T antigen is amino acid residue 128 (Lys→Asn). Transformation of precrisis rodent cells by pSVCT3 is negligible, whereas the frequency of transformation of established rodent cell lines by pSVCT3 is comparable to that of wild-type SV40. According to the model, in which transformation of precrisis cells involves the combined oncogenic action of both nuclear and cytoplasmic gene products, we predicted that pSVCT3 would localize in the cytoplasm of human cells and would therefore at most only partially and rarely transform precrisis human cells. We have found that pSVCT3 is able to transform precrisis human cells at high frequency. Furthermore, pSVCT3-transformed human precrisis cells relocalized T antigen to their nuclei. The relocalization of large T antigen was not dependent on cell growth. Wild-type and pSVCT3-transformed human cell lines both have about five copies of integrated SV40 DNA. SV40 virus-specific proteins, including the 100,000-molecular-weight super large T antigen, were expressed in pSVCT3-transformed human cells. Our results suggest that molecules in precrisis human cells, but not cells of other species, are able to complement the cytoplasmic-localization defect of the CT3 mutant large T antigen.

Simian virus 40 (SV40) encodes two early proteins, the large T and small t antigens. Large T antigen (T antigen) has been shown to be involved in the establishment of transformation, and in some cases, its continued expression is necessary as well for the maintenance of certain transformed phenotypes in nonpermissive cells (1, 15, 36, 37). Wild-type T antigen is localized in both the nucleus and the plasma membrane (8, 9, 19, 25, 30), with more than 95% of the total T antigen in the nucleus (19, 30).

T antigen has been shown to participate directly in many nuclear biochemical events, including initiation of viral DNA replication in lytically infected permissive cells (32), downregulation of its own synthesis (24, 33), activation of host ribosomal genes (29), stimulation of cellular DNA synthesis (11), provision of adenovirus helper function (6), high-affinity binding to specific DNA sequences around the SV40 origin of replication (34), in vitro ATPase activity (35), and formation of a stable nuclear complex with a cellular 53,000-molecular-weight phosphoprotein (53K phosphoprotein; 16).

The role of localization in transformation by T antigen has recently come under detailed study. A small fraction of wild-type T antigen is found on or under the plasma membrane of transformed cells, but its function is obscure (14, 27). Cytoplasmic-localization mutants of T antigen prevent its nuclear localization, but do not entirely suppress its capacity to transform established cells.

In rodents, wild-type SV40 T antigen is a complete oncogene product, with the ability to transform precrisis cells. SV40(ct)-3 appears to be a partial oncogene by comparison. SV40(ct)-3 is completely defective for the transformation of precrisis baby rat kidney cells (20, 21). The frequency of transformation of precrisis mouse embryo fibroblasts by this mutant is negligible by both the low-serum focus formation assay and the assay of anchorage-independent growth. In contrast, transformation of established mouse and rat cell lines by the cytoplasmic T antigen mutant of SV40 can occur. This second-step transformation occurs in the absence of detectable amounts of nuclear T antigen (21). Furthermore, transformation of established rodent cells by either the SV40-adenovirus hybrid mutant SV40(ct)-3 or *d*10, a point mutant of similar phenotype generated by mutagenesis, occurs at an efficiency comparable to that of the wild-type SV40 (13, 21).

In transformed established rodent cells, the T antigen of the cytoplasmic-localization mutant of SV40, SV40(ct)-3 (3, 17, 18) is accumulated in the cytoplasm instead of being transported to the nucleus. The mutant phenotype results from a single amino acid change: at amino acid 128 of T antigen (20), a positively charged lysine is replaced by a neutral amino acid, asparagine, which interrupts a stretch of five positively charged amino acids. Cytoplasmic T antigen mutants showing the same phenotype have also been generated by mixed oligonucleotide mutagenesis of the same part of the SV40 early region (13).

These data, and the overall model proposing that the transformation of precrisis cells involves the combined oncogenic action of both a nuclear immortalizing gene product (such as adenovirus E1a protein, *myc*, or polyomavirus T antigen) and a cytoplasmic protein (*ras* or polyomavirus middle t antigen; 38), suggested that the cytoplasmic mutant of SV40 would only partially or rarely transform precrisis human cells. Contrary to this expectation, we found that primary human cells were transformed by the cytoplasmic mutant of SV40. Furthermore, transformed human precrisis cells relocalized some T antigen to their nuclei.

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MATERIALS AND METHODS

Cells. CCDSK141 precrisis human cells were provided by M. Lipkin (Sloan-Kettering Institute for Cancer Research). C139, an origin-defective SV40-transformed human cell line, was obtained from H. Ozer (Hunter College, New York, N.Y.). All cells were grown in Dulbecco modified Eagle medium containing 10% fetal calf serum (HyClone) at 37°C in an atmosphere of 10% CO_2. The cells were routinely checked for pleuropneumonialike organisms by the Hoechst staining method and were found to be negative.

Plasmids. pSVCT3 was provided by J. Butel. The construction of pSVRIori-minus has been described earlier (4).

DEAE-dextran transfection of human cells. We used a slight modification of the DEAE-dextran method developed for monkey cells by Rio and Tjian (25). Human cells were plated at a density of 1×10^6 to 2×10^6 per 100-mm plate 24 h prior to transfection. Each plate was covered with 1 ml of a mixture containing 0.5 mg of DEAE-dextran and 5 μg of uncut plasmid DNA in Dulbecco modified Eagle medium. The plates were incubated at 33°C for 30 min with rocking every 5 min. Fresh medium with 0.1 mM chloroquine was then added. After 6 to 8 h of incubation at 37°C, the cells were rinsed twice with Dulbecco modified Eagle medium and fed with fresh growth medium. Three to five days after transfection, the cells were plated out at about 2×10^5 cells per 60-mm petri dish. Four to six weeks later, dense foci arising against a background of flat cells were picked with steel cloning rings for further analysis.

Immunofluorescent T antigen stain. The immunofluorescence staining of SV40 T antigen of infected culture at 48 h after transfection and of cloned dense foci was performed with monoclonal antibody 416 (10) as previously described.

Plating efficiency and serum requirement of transformants. For determination of plating efficiency, cells were plated out at 10^3 and 10^2 cells per 60-mm petri dish. Colonies were fixed, stained with crystal violet stain, and scored at day 10. To assay for serum requirement, cells were plated out at 5×10^4 per 60-mm plate (2×10^3 cells per cm^2) in either 10 or 1% fetal calf serum, and sister plates were counted by hemacytometer every 2 days for 10 to 12 days.

DNA replication assay. The intracellular accumulation of replicated viral DNA was assayed by isolation of low-molecular-weight DNA by the Hirt procedure (12) 72 h after superinfection of BSC-1, pSVCT3-transformed human cells, or pSVRIori-minus-transformed human cells. After digestion with *Dpn*I to eliminate nonreplicated input plasmid DNA, DNA was separated on a 0.8% agarose gel, transferred to GeneScreen Plus, and hybridized to an SV40 DNA probe labeled with ^{32}P by nick translation.

Analysis of integrated SV40 DNA. Genomic digests and Southern blotting were carried out as previously described (2, 31). Tandem repeats of viral DNA were identified by digesting the high-molecular-weight transformant DNA with several enzymes that cut the SV40 DNA in one place. Each digest was run in a separate, parallel gel lane. Comigrating fragments detected by ^{32}P-labeled SV40 DNA probe often represent viral DNA repeats, since a tandem duplication of a region of SV40 containing two or more sets of restriction enzyme sites creates same-size fragments when the DNA is cut with any of those enzymes (2). 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 kilobases. Although it is possible that fragments detected in this way represent some other structure, more-detailed work has shown that at least 80% of the fragments identified by this method in SV40-transformed mouse cell lines do in fact represent tandem partial SV40 duplications (2).

TABLE 1. Transformation frequency of CCDSK141

Passage	DNA source	No. of dense foci/μg of input DNA
Early[a]	pSVRIori-minus	4.2 ± 1[b]
Late[c]	pSVRIori-minus	2.3 ± 2
Early	pSVCT3	3.8 ± 3
Late	pSVCT3	1.3 ± 1
Early	pBR322	<0.01
Late	pBR322	<0.01

[a] Doubling 10 to 15.
[b] Mean and standard deviation; six experiments per point.
[c] Doubling 15 to 25.

Immunoprecipitation of viral protein. [^{35}S]methionine labeling, extraction, and immunoprecipitation were done as previously described (5).

RESULTS

pSVCT3-transformed precrisis human fibroblast cells. Rodent cells are transformed by wild-type SV40 at high efficiency. In this study, we used the origin-defective SV40 plasmid pSVRIori-minus as the source of wild-type T antigen for comparison with the transforming efficiency of the cytoplasmic-localization mutant T antigen of pSVCT3 because origin-plus wild-type SV40 has the ability to replicate autonomously in semipermissive human cells. Origin-defective wild-type SV40 has a high transformation efficiency in human cells and no background of SV40 replication (28, 37). Transformations were carried out with SV40(ct)-3 DNA cloned at the *Bam*HI site in plasmid pBR322 (21) and with wild-type pSVRIori-minus DNA cloned at the *Eco*RI site in pBR322 (4).

CCDSK141 primary human cells were transfected with either DNA by the DEAE-dextran method (25). Immunofluorescence assay of T antigen at 48 h posttransfection showed 0.07 ± 0.01% T- antigen-positive cells per μg of input DNA. pSVRIori-minus and pSVCT3 did not differ in their abilities to induce T antigen at 48 h. pSVRIori-minus DNA transfection generated CCDSK141 cells with T antigen in nuclei at 48 h, whereas pSVCT3 DNA transfection yielded some cells with T antigen solely in the cytoplasm (95 to 98% of positive cells) and others with T antigen in both the nuclei and the cytoplasm (2 to 5% of positive cells). The detection of nuclear T antigen in pSVCT3-infected cells by 48 h after infection was surprising, since pSVCT3 T antigen has not been reported to be transported to the nucleus and reversion at such a high frequency has not been observed after acute infection of monkey cells (data not shown; 20).

Four to six weeks after infection, transformed foci were scored, counted, or cloned from dense regions against backgrounds of untransformed cells. The frequency of transformation of CCDSK141 cells by pSVRIori-minus and pSVCT3 is shown in Table 1. Each datum point is the mean and standard deviation of six experiments. Low but similar numbers of dense foci arose from transfection with either type of DNA.

Human fibroblasts have a finite lifetime in culture. At later passages, the frequency of transformation decreased for both types of DNA (Table 1). No dense foci were seen in the cells transfected with control plasmid pBR322 (Table 1).

pSVCT3 T antigen in cytoplasm and nucleus in cloned transformed human cell lines. The cytoplasmic mutation CT3

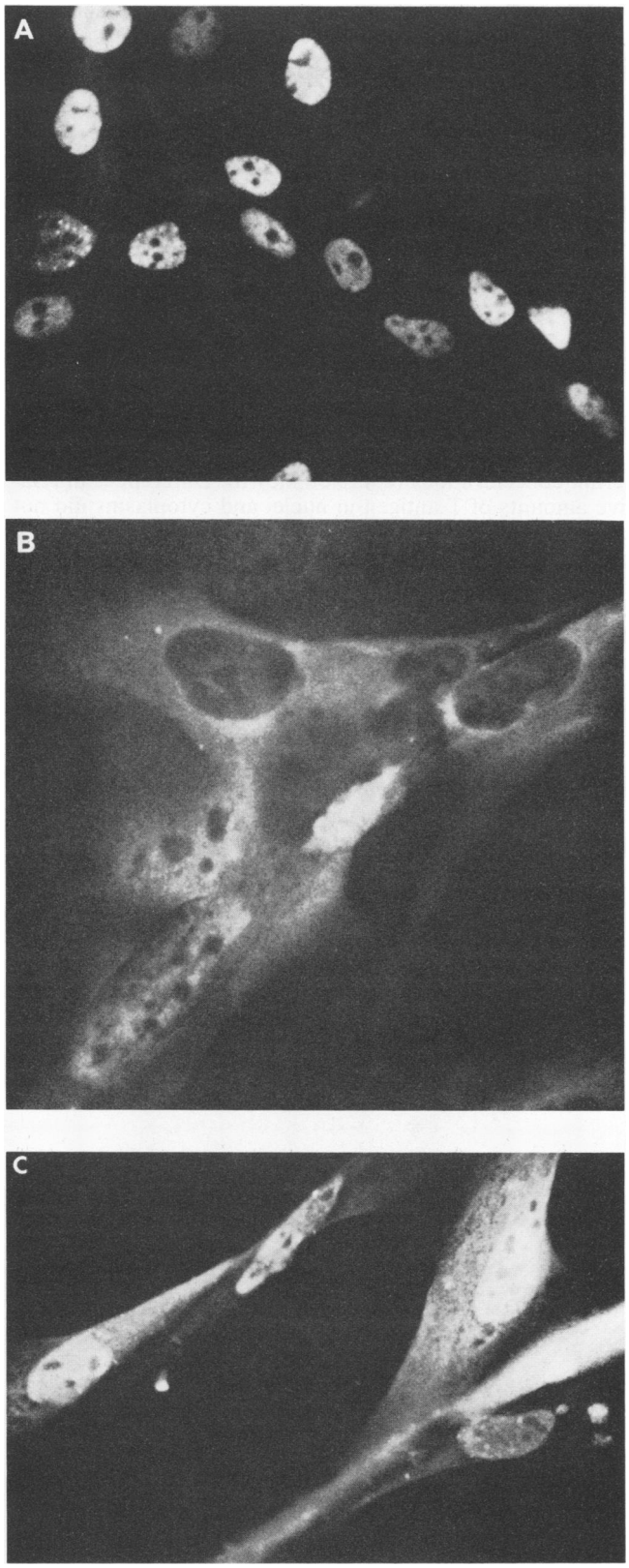

FIG. 1. Intracellular distribution of SV40 T antigen in pSVRIori-minus-transformed (A) and pSVCT3-transformed (B and C) human cells. Only nuclear fluorescence is present in panel A, whereas panel B shows nuclear or cytoplasmic T antigen staining, and panel C has T antigen in both places.

TABLE 2. Distribution of T antigen

Cell species	Clones and subclones	Percentage of T antigen in:		
		Nucleus	Cytoplasm	Both nucleus and cytoplasm
Human	pSVRIori-minus-1-2/5	100	0	0
	pSVRIori-minus-2-2/12	100	0	0
	pSVRIori-minus-2-2/12-3	100	0	0
	pSVRIori-minus-2-2/12-4	100	0	0
	pSVRIori-minus-2-2/12-6	100	0	0
	pSVCT3-2/7	34	13	53
	pSVCT3-2/7-5	43	3	54
	pSVCT3-2/7-8	25	7	68
	pSVCT3-2/7-9	59	13	28
	pSVCT3-2/5	40	16	44
	pSVCT3-2/5-4	54	4	42
	pSVCT3-2/5-5	55	7	38
Mouse	SVCT3-3T3	0	100	0
Monkey	SVCT3-BSC-1	0	40[a]	0

[a] The results of distribution of T antigen in monkey BSC-1 cells at 48 h posttransfection with SVCT3 plasmid DNA, unlike the human and mouse transformants, which were isolated as dense foci 3 to 5 weeks after transfection with SVCT3 DNA.

prevents nuclear localization of T antigen in mouse, rat, and monkey cells, both in acute infection and after transformation (18, 20). Human cells were infected with pSVRIori-minus and pSVCT3 DNA. Dense foci were cloned and examined for T antigen by immunofluorescence. All pSVRIori-minus transformed human cell lines showed T antigen in their nuclei. Each clone of pSVCT3 transformant had a fraction of cells with T antigen completely in the cytoplasm, a fraction with nuclear T antigen, and a fraction with T antigen in both places (Fig. 1).

Each pSVCT3 transformant clone showed a different distribution of T antigen (Table 2). To rule out the possibility that a "clone" was not a pure clone but rather a mixed population of descendants of infected cells, subcloning was carried out. Table 2 shows the results of the distribution of T antigen in these subclones. pSVRIori-minus subclones still maintained 100% T-antigen-positive nuclei, whereas subclones of pSVCT3 transformants showed distributions of T antigen similar to the distributions of the parental clones (Table 2). Culture conditions did not affect the distribution of pSVCT3 T antigen in human cells. Concentrations of serum in the growth medium from 0 to 10% and cell density from 1/10 confluent to 3× confluent permitted pSVCT3 T antigen to be localized in the nuclei of transformed human cells. Comparable distributions of nuclear T antigen and cytoplasmic T antigen in infections occurred in HeLa cells, as well as in human precrisis cells from skin biopsies of seven individuals (data not shown).

Identical growth properties of pSVRIori-minus- and pSVCT3-transformed human cells. We next compared the growth properties of pSVCT3 and pSVRIori-minus human transformants to determine whether human cells transformed by the mutant T antigen were different in phenotype from wild-type transformants. Transformants originally selected as dense foci in 10% serum were grown in 10 or 1% serum (Fig. 2). Both pSVRIori-minus and pSVCT3 transformants grew in either 10 or 1% serum at rates comparable to that of SV101, a well-characterized, SV40-transformed mouse 3T3 cell line (Table 3). The untransformed parent CCDSK141 grew more slowly and reached a

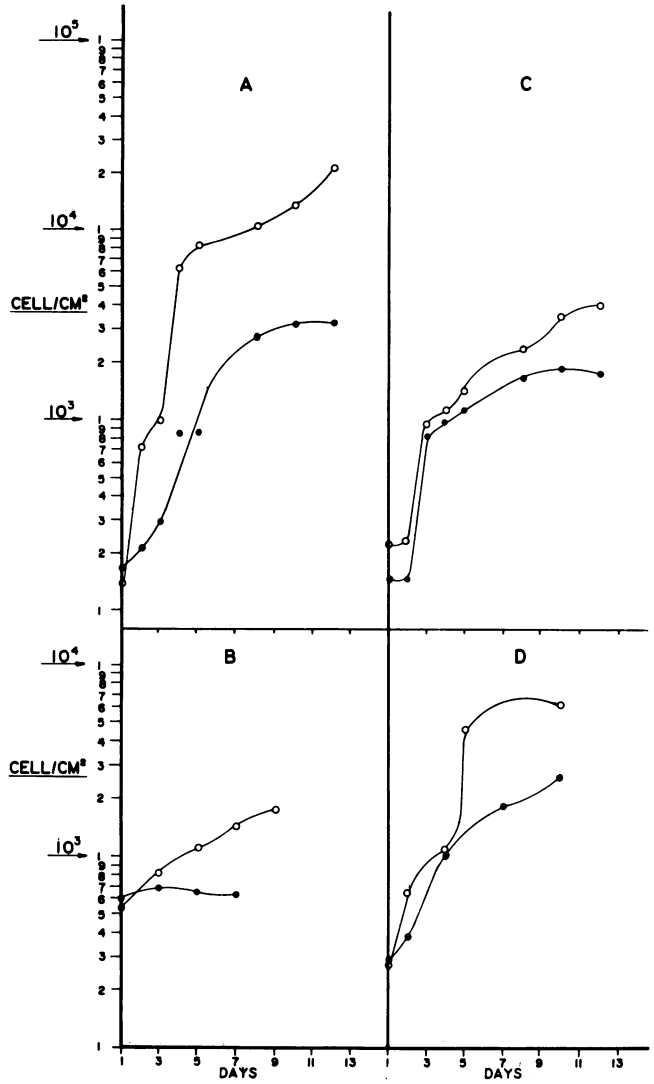

FIG. 2. Growth properties of pSVRIori-minus- and pSVCT3-transformed cells. (A) SV101-SV40-transformed Swiss 3T3 mouse cells; (B) CCDSK141 normal human cells; (C) pSVRIori-minus-transformed CCDSK141 cells; (D) pSVCT3-transformed CCDSK141 cells. ○, 10% serum; ●, 1% serum.

lower saturation density than either wild-type or pSVCT3 human transformants in high serum concentrations and did not grow at all in low serum concentrations (Fig. 2, Table 3). Other pSVRIori-minus-or pSVCT3-transformed clones showed comparable growth patterns in both high and low

TABLE 3. Growth parameters of human cells

Cell line	Doubling time[a] (days) in:		Saturation density (10^4 cells/cm^2) in:	
	10% serum	1% serum	10% serum	1% serum
CCDSK	4.0	>10	1.6	0.66
CCD-CT3-2/5	2.2	3.1	5.4	2.2
CCD-ori-2/12	2.5	2.6	3.7	1.8
SV101	1.4	2.1	17.5	3.2

[a] CCDSK was transformed at doubling 10 to 15. Experiments were carried out during doublings 25 to 30 of transformed human clones.

TABLE 4. Growth properties of pSVRIori-minus- and pSVCT3-transformed precrisis human cells

Cell line	No. of adherent colonies per		Plating efficiency[a]	Anchorage-independent growth[b]
	10^3 cells	10^2 cells		
CCDSK	1	<0.01	<0.01	0
CCD-ori-2/5	4.6	0.2	0.33	0
CCD-ori-2/12	11	0.8	0.95	0
CCD-CT3-2/5	14	0.8	1.1	0
CCD-CT3-2/7	34.4	2.4	2.9	0
SV101 (mouse)			0.3[c]	14 ± 2.7
C139	10	0.7	0.85	1.5 ± 0.6

[a] Average number of colonies per 100 cells plated.
[b] Number of colonies >0.2 mm in diameter per 10^4 cells plated in agarose.
[c] Data from Pollack and Vogel (23).

serum concentrations (data not shown). Differences in relative amounts of T antigen in nuclei and cytoplasms did not correlate with differences in growth properties among the pSVCT3-transformed clones.

The efficiencies of anchorage-dependent colony formation of pSVRIori-minus and pSVCT3 human transformants were higher than that of untransformed CCDSK141 (Table 4). It has been reported that most SV40-transfected human fibroblasts will not grow in agarose without anchorage (28). None of the SV40-transformed human cells we isolated could grow in agarose (Table 4). Two positive controls were used: SV101 mouse cells grew in agarose, as did C139, an origin-defective SV40-transformed human cell line derived from a single colony growing in agarose (D. Neufeld and H. Ozer, personal communication; Table 4). We conclude that within the limits of the small number of clones examined, the phenotype of pSVCT3-transformed human cells is not dis-

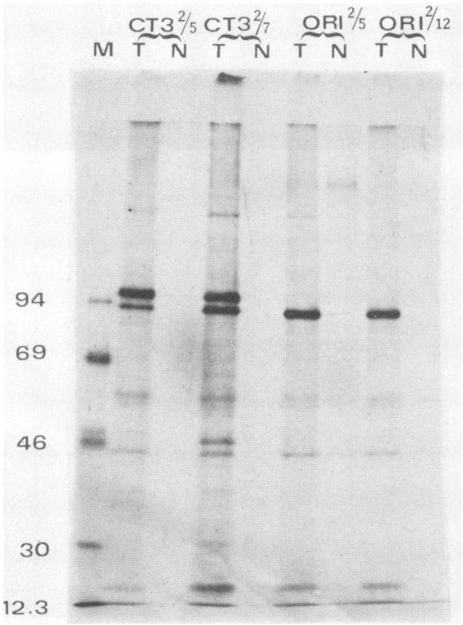

FIG. 3. Autofluorogram of immunoprecipitations of [^{35}S]methionine-labeled cell extracts from pSVCT3-2/5, pSVCT3-2/7, pSVRI ori-minus-2/5, and pSVRIori-minus-2/12 human transformants. T, Tumor serum; N, normal serum. Numbers indicate the sizes (in kilodaltons) of protein markers.

tinguishable from that of pSVRIori-minus-transformed human cells.

Production of 94K T and 100K super T antigens in pSVCT3 transformants. The 100K super T antigen has been detected in all origin-plus, wild-type SV40-transformed mouse cells (4). No origin-minus-transformed rodent cell lines produce detectable amounts of the 100K super T antigen. Transformed human clones, isolated after transfection by pSVCT3 or pSVRIori-minus plasmid, were analyzed by immunoprecipitation. The cells labeled with [^{35}S]methionine extracts were immunoprecipitated either with normal hamster serum or with hamster antitumor serum and separated on 10 to 20% gradient polyacrylamide gels. All clones examined carry the expected T antigen and small t antigen and also the nonviral 53K protein which binds tightly to T antigen (Fig. 3; 16). In addition to these proteins, pSVCT3 transformants also expressed the 100K super T antigen (Fig. 3). These results extend to human cells the previous observation that wild-type pSVRI-transformed mouse cells express both 94K and 100K T antigens, while pSVRIori-minus mouse transformants express only the 94K T antigen (4). In addition, 100K T antigen expression can occur without a T antigen capable of the replication function.

Structure of SV40 DNA inserts in precrisis human cells transformed by pSVRIori-minus or pSVCT3 DNA. A functional T antigen and origin of replication are both required for the rearrangement of integrated polyomavirus DNA sequences (7). We have analyzed the integrated viral DNA in the pSVRIori-minus and pSVCT3 transformants, both of which lack replication function. DNA from both types of transformants was digested with four restriction enzymes, each of which cuts SV40 DNA in one place. Southern blot analysis shows that pSVRIori-minus and pSVCT3 transformants both have about 5 to 10 copies of integrated SV40 DNA (Fig. 4). Both types of transformants showed comparable formation of tandem arrays of integrated SV40 DNA, that is, bands that comigrate after digestion with different one-cut enzymes (Fig. 4; see Materials and Methods for a detailed description). Apparently, in human cells, as in mouse cells (G. Blanck, D. Li, E. Pomert, R. E. Pollack, and S. Chen, manuscript in preparation) but not rat cells (7), a functional origin of replication of SV40 is not needed for tandem repeat formation.

Replication function lacking in T antigen in pSVCT3-transformed human cells. Replication of SV40 requires a permissive cell species, a functional T antigen, and a functional origin of replication in the same cell. Human cells are semipermissive for SV40 replication. Therefore, superinfection of pSVCT3 transformants with pSVRIori-minus should result in replication of the integrated viral DNA, because the functional T antigen from pSVRIori-minus should activate the functional origin of replication of the integrated pSVCT3. To test this, we first transfected pBR, pSVRIori-minus, or pSVCT3 DNA into pSVCT3 human transformants. We then examined the cells for evidence of viral DNA replication.

Low-molecular-weight DNA was isolated at 72 h posttransfection, digested with *Dpn*I (a restriction enzyme that cleaves only DNA grown in bacteria), and analyzed in a Southern blot with ^{32}P-labeled SV40 DNA as a probe (2, 30). Only DNA that had replicated in the mammalian cells remained intact after treatment with *Dpn*I, whereas nonreplicated input plasmid DNA was digested to small fragments. In a control experiment carried out on BSC-1 monkey cells, only pSVRI, the wild-type SV40 DNA, showed replication. As expected, neither pSVRIori-minus nor pSVCT3 DNA was able to replicate in BSC-1 cells (data not shown). Replication of SV40 DNA occurred only when pSVCT3 transformants were superinfected with pSVRIori-minus DNA and not when the superinfecting DNA was pBR or pSVCT3 (data not shown). Apparently, pSVCT3 viral DNA integrated in transformed human cells retains its origin and also its characteristic inability to replicate in the absence of wild-type T antigen.

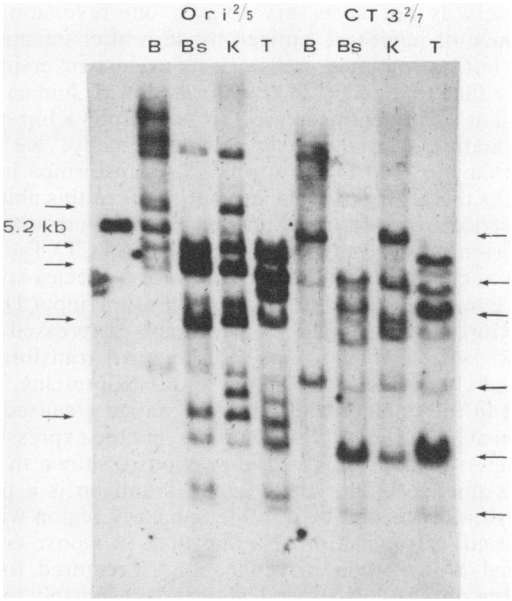

FIG. 4. Southern blot analysis of ori-minus-2/5 and pSVCT3-2/5 human transformants. DNA was cut with restriction enzymes that cut SV40 in only one place. B, *Bcl*; Bs, *Bst*XI; K, *Kpn*; T, *Taq*. Arrows indicate partial repeats (see Materials and Methods). kb, Kilobases.

DISCUSSION

The T antigen of pSVCT3, a mutant of SV40 that is defective for the transport of T antigen to the nucleus of monkey cells, transforms precrisis human cells as efficiently as the wild-type T antigen of pSVRIori-minus (28). Transformants isolated after transfection with pSVCT3 showed growth properties comparable to those of pSVRIori-minus-transformed clones. As early as 48 h after transfection with pSVCT3, a small percentage of human precrisis cells was found with T antigen in the nuclei. Stable pSVCT3-transformed clones always had a large fraction of cells with T antigen in the nucleus. Further subcloning of these clones did not reduce the percentage of nuclear T antigen cells. The distribution of T antigen was not related to the growth condition of the cells.

Because previous reports have placed CT3 in the cytoplasm of transfected and transformed rat, mouse, and monkey cells, it is reasonable to ask whether pSVCT3 DNA had undergone mutation with high frequency in human cells. The most direct way to rule out second-site mutation or back mutation would be to recover and then sequence the integrated pSVCT3 DNA from a human transformant, but such recovery is problematic because these transformed human cells contain more than one copy of SV40 DNA. It would be impossible to be sure that a given recovered copy of SV40 DNA was the one responsible for the transformation, whether or not it was reverted.

Recovery is not necessary to rule out reversion. The appearance of nuclear T antigen by 48 h after infection of human, but not monkey, cells argues against reversion, as does the fact that pSVCT3 transformation of human cells occurred at a frequency identical to that of pSVRIori-minus transformation in these experiments. Finally, we have shown that the viral DNA in pSVCT3-transformed human cells lacks the ability to replicate, but acquires this ability in the presence of wild-type T antigen from supertransfected pSVRIori-minus DNA. We conclude that pSVCT3 T antigen stays in nuclei of human cells because of a species-specific cellular event, not because of mutation of the input DNA.

pSVRIori-minus human transformants expressed only 17K and 94K T antigens. pSVCT3 human transformants expressed virus-specific 17K, 94K, and 100K proteins. Thus, a defect in the nuclear transport of T antigen, caused by a mutation at amino acid 128, did not prevent the expression of 100K super T antigen. We have previously shown that the coding sequence of the 100K super T antigen is a partial early region preceeded by a full-length early region with an intact origin of replication (22) and that, in mouse cells, a functional SV40 origin of replication is required for the generation of the 100K super T antigen. Presumably, origin-defective pSVRIori-minus-generated human transformants, like their mouse counterparts, cannot undergo the specific rearrangement of integrated DNA necessary for 100K super T production. The mutation of pSVCT3 at amino acid 128 (nucleotide 4434) is very close to the proposed cryptic splicing donor site (nucleotide 4424) for the generation of 100K protein (22). Nevertheless, the CT3 mutation does not affect the capacity of the integrated SV40 DNA to undergo rearrangement sufficient for the expression of 100K super T antigen to occur.

Our results support the general theory that a member of the nuclear-protein oncogene complementation group is needed for complete transformation of precrisis cells. It is not clear at present why cells of some species are able to transport cytoplasmic mutant T antigen to the nucleus and cells of other species are not. We have demonstrated that primary human cells, unlike primary rat cells, can be transformed by the T-antigen-transport-defective mutant of SV40. Species-specific molecules play an important role in determining the transformation potential of a given viral or oncogenic DNA. If pSVCT3-transformed human cells have not arisen by the second mutation of the pSVCT3 DNA, then comparison of pSVCT3-transformed human, monkey, mouse, and rat cells may provide a way to isolate the human cell factors capable of complementing the cytoplasmic-T-antigen defect.

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