MOLECULAR CLONING AND CHARACTERIZATION OF ENDOGENOUS SV₄₀ DNA FROM HUMAN HBL-100 CELLS

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The human HBL-100 cell line harbours SV₄₀ DNA integrated in tandem at a unique site. The SV₄₀ T-antigen expressed in these cells is defective in a function essential to the replication of the viral genome. The integrated SV₄₀ sequences were molecularly cloned in a bacteriophage, and a subclone (plasmid pSVHBI) containing a complete SV₄₀ DNA was isolated. As compared to SV₄₀ wild-type strain 776, sequence analysis of pSVHBI early region revealed the presence of several DNA alterations. Among these, a point mutation at position 3199, predicting a change at amino-acid 540 of arginine to isoleucine, was shown by marker rescue to be responsible for the deficiency of T-antigen. This novel mutation further delimits one of the T-antigen domains involved in SV₄₀ DNA replication. Transfection experiments demonstrated that the transforming activity of the SV₄₀ genome from HBL-100 cells is still preserved. Moreover, several transformed human cell clones thus obtained could be permanently established in culture.

The epithelial HBL-100 cell line, established *in vitro* from the milk of an apparently healthy woman, exhibits characteristics of transformation from the very beginning and evolves during *in vitro* maintenance until it becomes tumorigenic in nude mice.

In a previous publication (Caron de Fromentel *et al.*, 1985) we reported the unexpected finding that HBL-100 cells harbour SV₄₀ DNA integrated in tandem at a unique site in the cellular genome. By *in situ* hybridization, the integration site of the viral DNA was shown to be located on human chromosome 15, at band 15q24 (Marlhens *et al.*, 1988). It seems likely that virus infection occurred *in vivo*, possibly by SV₄₀ administered inadvertently with a polio virus vaccine (Caron de Fromentel *et al.*, 1985).

With regard to the SV₄₀ T-antigen expressed in HBL-100 cells, we have previously shown (Caron de Fromentel *et al.*, 1985) that it can bind to the SV₄₀ origin of replication but that this property is not sufficient to permit the rescue of the integrated viral genome after fusion with permissive CV-1 cells, although rescue takes place upon fusion with permissive COS-7 cells which harbour a replication-competent SV₄₀ T-antigen gene. This indicates that this protein has a defect which does not interfere with its DNA-binding properties but affects at least one function essential to the replication of the viral genome.

In order to address this issue, as part of a project concerning the biological and molecular characterization of the HBL-100 cell line, we have molecularly cloned the endogenous SV₄₀ DNA, sequenced its early region and identified the alteration responsible for the lack of viral DNA replication. In addition, we have determined the capacity of the cloned viral genome to transform human and rodent cells in culture.

MATERIAL AND METHODS

Nomenclature

The nucleotide numbering system used for the SV₄₀ genome is that of Buchman *et al.* (1982) and is based on the 5,243 base-pair (bp) genome, with the unique BglI palindrome centred on position 0/5243. Specific nucleotide positions in the cloned inserts of plasmids pSVHBI and pSVHB2 are referred

to by their analogous positions in the genome of the wild-type SV₄₀ strain 776.

Cell cultures

HBL-100 is an epithelial cell line established *in vitro* from the milk of an apparently healthy woman (Polanowski *et al.*, 1976; Gaffney, 1982); CV-1 is an established line of epithelial monkey kidney cells, fully permissive for lytic growth of SV₄₀ (Manteuil *et al.*, 1973); ICIG-7 is a diploid fibroblast cell line with a limited *in vitro* life span, derived from normal human embryonic lung (Macieira-Coelho and Azzarone, 1982) and semi-permissive for SV₄₀ infection; 3T3-Vill is a contact-inhibited Swiss mouse embryonic cell line established in our laboratory and non-permissive for SV₄₀ infection.

All these lines were grown routinely in Eagle's minimal essential medium supplemented with 10% newborn calf serum (MEM 10).

DNA preparation and hybridization

Preparation of high-molecular-weight cellular DNA and of SV₄₀ DNA, electrophoresis in agarose gels, nick-translation of DNA probes and blot hybridization were performed as previously described (Caron de Fromentel *et al.*, 1985).

Bacterial strains, bacteriophages and plasmids

All the procedures involving bacteriophages and plasmids were carried out according to Maniatis *et al.* (1982). *Escherichia coli* strains Q359 and Q358 (Karn *et al.*, 1980), used as recipients for bacteriophages, were a gift from Dr. O. Brison (IGR, Villejuif, France). Bacterial strain AG1, derived from *E. coli* DH1 (Hanahan, 1983) and used for plasmid preparations, as well as bacteriophage λEMBL3 (Frischauf *et al.*, 1983) were purchased from Stratagene, San Diego, CA. Plasmids pUC13 (Messing and Vieira, 1982) and pSVTneoZ (Sarasin *et al.*, 1987), which contains a functional early region from wild-type SV₄₀ DNA, were provided by Dr. M. James (IRSC, Villejuif, France). Plasmid pLASwt (Daya-Grosjean *et al.*, 1987), containing an origin-minus SV₄₀ DNA early region and expressing a fully functional T-antigen, was a gift from Dr. L. Daya-Grosjean (IRSC, Villejuif, France). Packaging and sequencing kits were purchased from Stratagene. Bacterial strain DH5αF' and plasmid pTZ18R (plus helper phage M13K07) used for marker rescue experiments were purchased from BRL, Gaithersburg, MD, and Pharmacia, Uppsala, Sweden, respectively.

Molecular cloning of integrated SV₄₀ DNA

High-molecular-weight genomic DNA from HBL-100 cells was digested to completion with BglII. The DNA fragments were ligated to the arms of λEMBL3 bacteriophage vector, generated by cleavage with BamHI. Packaging and propagation of the DNA recombinants in *E. coli* strain Q359 were carried out by standard procedures (Maniatis *et al.*, 1982). This

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partial library was screened by plaque hybridization using *in vitro* nick-translated SV₄₀ DNA as a probe.

DNA sequencing

Sequencing was performed by the dideoxy-termination method (Sanger *et al.*, 1977) with alkali-denatured plasmid DNA templates (Hattori and Sakaki, 1986) and deoxyadenosine 5'- α -(³⁵S)thiotriphosphate (Amersham, Little Chalfont, UK). In order to sequence the SV₄₀ early region contained in plasmid pSVHB1 (Fig. 1), 2 strategies were followed. (1) pSVHB1 was doubly digested with *Sac*I and *Nco*I generating a *Sac*I-*Nco*I fragment containing the pUC13 sequences and the complete SV₄₀ early region. Unidirectional deletions were introduced into the latter, from the *Nco*I site, by the method of Henikoff (1984), the only difference being that mung bean nuclease was used instead of S1 nuclease. The deleted fragments were circularized by ligation so that the deletion break points were positioned next to the M13 reverse primer site from pUC13. Plasmids thus obtained, with deletions of variable length into the SV₄₀ sequences, were cloned and amplified. The sequence of overlapping DNA stretches of 200–300 nucleotides was then determined using the M13 reverse primer (Stratagene). (2) Confirmation of the results was obtained by direct sequencing of pSVHB1 using a selected set of 17-mer oligonucleotides synthesized with an Applied 380 B apparatus (BRL) and purified on an HPLC column by Dr. J. Armier (IRSC, Villejuif, France).

Marker rescue

Four synthetic oligonucleotides were prepared by Dr. J. Armier as described above: h4wt (5'-TTTACAAATCTGGCCTGCA); h4mut (5'-TTTACAAATATGGCCTGCA); h5wt (5'-AAACTCCAATCCCATAGC); h5mut (5'-AAACTCCAGTCCCATAGC). A plasmid designated pTSVHB was constructed in order to use a procedure based on the method of Zoller and Smith (1983) for oligonucleotide-directed mutagenesis. This plasmid was obtained by inserting the SV₄₀ early region isolated from pSVHB1 by digestion with *Kpn*I and *Eco*RI into the pTZ18R vector. This vector bears an *f*1 phage origin of replication

which is required for the synthesis of single-stranded DNA in the presence of M13K07 helper phage. Single-stranded DNA was purified following M13K07 helper phage infection of *E. coli* DH5 α F' carrying the pTSVHB plasmid. One pmole of single-stranded template was annealed with appropriate pairs of phosphorylated oligonucleotide primers (h4mut and h5mut; h4wt and h5mut; h4mut and h5wt; h4wt and h5wt; 5 pmole of each oligonucleotide). The resulting hybrid DNAs were then filled in with the Klenow DNA polymerase and ligated. A small aliquot of each reaction mixture was used to transform AG1 cells. The supercoiled plasmid DNAs thus obtained were purified and transfected into subconfluent CV-1 cells using the DEAE-dextran method of Kimura and Dulbecco (1972). Three days later, low-molecular-weight DNA was selectively extracted from each culture by the procedure of Hirt (1967). The purified DNA was digested with *Kpn*I and *Dnp*I and analyzed by Southern blot hybridization using *in vitro* nick-translated pSVHB1 as a probe.

Growth in soft agar

The method described by MacPherson and Montagnier (1964) was used.

Transforming activity of SV₄₀ DNA

DNA transfection was carried out using the calcium phosphate co-precipitation method (Wigler *et al.*, 1978). Human ICIG-7 cells (pass. 20–28) or mouse 3T3-Vill cells (pass. 33) were seeded into 100-mm Falcon dishes at a concentration of 1.5×10^6 cells/10 ml MEM 10/dish. The next day, an equivalent number of cultures was transfected with 1 ml/culture of DNA-calcium phosphate co-precipitate containing either 40 μ g of calf thymus DNA, as a control, or 1 μ g of SV₄₀ DNA-containing recombinant plus 40 μ g of carrier calf thymus DNA. After 6 hr at 37°C, the medium was removed and 10 ml of fresh MEM 10/dish were added. Two days later, the cells were trypsinized and seeded either in liquid medium or in soft agar at a concentration of 2×10^5 cells/60-mm Falcon dish. Transformed foci or colonies were scored after 3–4 weeks' incubation.

FIGURE 1 – Physical maps of SV₄₀ DNA integrated sequences and cloned inserts. The map at the top shows the structure of the SV₄₀ DNA sequences (white areas) integrated within the genome of HBL-100 cells (black areas), as determined by Southern blotting. λEHBBg is a phage recombinant containing the whole integrated viral DNA with flanking cellular sequences inserted in λEMBL3 DNA (dotted areas). pSVHB2 and pSVHB1 are recombinant plasmids harbouring fragments from the λEHBBg insert subcloned in pUC13 (shaded areas). The line drawn below the pSVHB1 map delimits the early SV₄₀ region subjected to sequence analysis.

Immunofluorescence

Expression of SV₄₀-specific T-antigen was detected by indirect immunofluorescence (Wicker and Avrameas, 1969), with Syrian hamster anti-SV₄₀ tumour serum and fluorescein isothiocyanate-conjugated rabbit antiserum to hamster γ -globulin.

RESULTS

Molecular cloning of integrated SV₄₀ DNA

Previous results had shown that the SV₄₀ T-antigen expressed in HBL-100 cells is affected in a function essential to the replication of the viral genome (Caron de Fromentel *et al.*, 1985). In order to identify the structural alteration responsible for the inactivation of this function, we have molecularly cloned the SV₄₀ DNA integrated in tandem (Fig. 1). Previous data had shown that the viral DNA sequences were contained in a unique 12-kbp *Bgl*II fragment of HBL-100 genomic DNA (Caron de Fromentel *et al.*, 1985). Thus, 0.6 μ g of HBL-100 DNA were digested with *Bgl*II and ligated to 1.5 μ g of *Bam*HI-cleaved λ EMBL3 phage DNA. Approximately 250,000 recombinants were obtained, 100,000 of which were screened with an SV₄₀ DNA probe leading to the isolation of one positive recombinant (λ EHBBg). Upon digestion with various restriction enzymes and electrophoresis on agarose gels, the λ EHBBg DNA displayed the expected band patterns. Two overlapping fragments of about 5.2 kbp, obtained by cleavage with either *Bam*HI or *Eco*RI, were isolated and subcloned in the pUC13 plasmid vector (Fig. 1). The 2 recombinant plasmids pSVHB1 and pSVHB2 were extensively studied by restriction enzyme mapping. It was established that pSVHB1 harbours a complete SV₄₀ genome whereas pSVHB2 contains an incomplete viral genome with a truncated early region flanked by a cellular sequence of about 2.1 kbp. The junction between the viral and cellular sequences has been located at nucleotide 4037 interrupting the coding sequence of T-antigen at the 260th codon, which would lead to a truncated protein of about 34 kDa. When compared to the wild-type SV₄₀ strain

776 by restriction enzyme mapping, a noticeable difference resides in the size of the *Hind* III C DNA fragment from both pSVHB1 and pSVHB2 inserts. Refined analysis of both plasmids doubly digested with *Hind* III and *Kpn* I showed that the fragment containing the SV₄₀ origin of replication has a size of approximately 290 bp whereas the same wild-type fragment is 366 bp long. This size difference (approx. 70 bp) would be consistent with the presence, in the strain of SV₄₀ which originally infected the HBL-100 cells, of a single copy of the 72-bp enhancer which is found duplicated in strain 776 DNA. This was actually confirmed by sequence analysis of this region in pSVHB1 (data not shown).

Sequencing of SV₄₀ early region contained in pSVHB1

The SV₄₀ genomic portion contained in pSVHB1, corresponding to the complete early region, encompassing nucleotides 150 to 0 and 5243 to 2600 in the sense of the early RNA was sequenced and compared to that of the wild-type SV₄₀ strain 776. As shown in Figures 2 and 3a, 11 nucleotide substitutions were found, 3 of them (h1, h2 and h3) altering the amino-acid sequence of small t-antigen and 3 of them (h4, h5 and h6) modifying the C-terminal part of large T-antigen. In addition, 2 changes which involve several nucleotides but which do not shift the reading frame were observed in the T-antigen coding sequence, proximal to the stop codon: (1) an insertion of 9 bp in the region around nucleotide 2795, which had already been reported for SV₄₀ strains 777 (Manos and Gluzman, 1985) and LP (Feunten *et al.*, 1981); (2) a deletion of 6 bp in the region around nucleotide 2770, not previously described (Figs. 2 and 3b). Two of the conservative nucleotide changes mentioned above have been observed at position 3755 for strain 777 and at position 2757 for other SV₄₀ strains (Manos and Gluzman, 1985). To our knowledge, none of the other modifications listed in Figure 3a have been described as yet. The 3 point mutations h1, h2 and h3 affecting the amino-acid sequence of small t-antigen would be without consequence for SV₄₀ replication since mutants with deletions covering this region display a normal lytic cycle (Bouck *et al.*, 1978;

FIGURE 2 – Location of the mutations identified by sequence analysis in the SV₄₀ early region of pSVHB1. The black line at the top represents the nucleotide sequence of the SV₄₀ early region. The positions of the initiation and termination codons for small t- and large T-antigens are indicated. ORI corresponds to the origin of viral DNA replication. Below the black line, the small arrows show the positions of the single nucleotide substitutions, and the large arrow that of both the insertion of 9 bp and the deletion of 6 bp (see ‘‘Results’’). The uppermost box represents the coding sequence for small t-antigen, where the 3 predicted amino-acid changes h1, h2 and h3 have been located. The 2 lower boxes delimit the coding region for large T-antigen, interrupted by the sequence spliced out in the corresponding transcript. h4, h5 and h6 show the position of 3 amino-acid substitutions, INS, that of a 3 amino-acid insertion and DEL, that of a 2 amino-acid deletion. Below the 2 lower boxes are indicated the positions of known large T-antigen mutations (see References cited in Gish and Botchan, 1987) that inactivate SV₄₀ DNA replication and which are clustered in 3 separate domains of the protein. Portions of large T-antigen that encode various functions of this protein are bracketed. Nucleotide and amino-acid numbering is based on that of SV₄₀ wild-type strain 776.

A POINT MUTATIONS

nucleotide		amino acid		name
N°	change	N°	change	
4879	ATG to ATA	95	Met to Ile	h1
4853	GCA to GTA	104	Ala to Val	h2
4839	GCT to ACT	109	Ala to Thr	h3
4750	TGC to TGI		conservative	
3930	CAG to CAA		conservative	
3755	AAT to AAC		conservative	
3199	AGA to ATA	540	Arg to Ile	h4
3117	AGA to AGG		conservative	
2950	ATT to ACT	623	Ile to Thr	h5
2918	GAT to AAT	634	Asp to Asn	h6
2817	TCC to TCI		conservative	
2757	TAC to TAI		conservative	

B INSERTION-DELETION

	2801		2760
wt 776	GCC.CCT.CAG.	.TCC.TCA.CAG.TCT.GTT.CAT.GAT.CAT.AAT.CAG.CCA.	
	Ala Pro Gln	Ser Ser Gln Ser Val His Asp His Asn Gln Pro	
	673		686
pSVHB1	GCC.CCT.CAG.CCC.TCA.CAG.TCC.TCA.CAG.TCT.GTT.CAT.GAT.	.CAG.CCA.	
	Ala Pro Gln Pro Ser Gln Ser Ser Gln Ser Val His Asp	Gln Pro	

FIGURE 3 – (a) Single nucleotide and amino-acid changes found in the early region of SV₄₀ cloned in pSVHB1. (b) Partial sequence of pSVHB1, compared to the homologous region of SV₄₀ strain 776, showing the insertion of 9 nucleotides coding for 3 amino-acids, and the deletion of 2 nucleotides coding for 2 amino-acids.

Feuntun *et al.*, 1978; Shenk *et al.*, 1976; Sleight *et al.*, 1978). One deletion mutant (dl 2199) encompassing the h6 modification does not show any significant difference in virus yield on CV-1 cells, as compared to wild-type SV₄₀ (Pecceu *et al.*, 1987). The deletion of 6 bp around nucleotide 2770 would not be expected to affect viral DNA replication since 2 deletion mutants (dl 1066 and dl 1140) overlapping this region are positive for this function (Pipas *et al.*, 1983). Consequently, we were left with the h4 and h5 mutations as being possibly responsible for the defectiveness of the T-antigen expressed in HBL-100 cells. In order to solve this question we performed marker rescue experiments.

Marker rescue with synthetic oligonucleotides

Marker rescue experiments were carried out with synthetic oligonucleotides covering the sites of the h4 and h5 mutations. As described in "Material and Methods", for each of these sites 2 oligonucleotides were synthesized, one with the wild-type sequence (h4wt, h5wt) and the other with the mutated

sequence (h4mut, h5mut). Single-stranded pTSVHB plasmid DNA, containing the SV₄₀ early region from pSVHB1, was simultaneously annealed with each of the following pairs of oligonucleotides: h4wt and h5mut, h4mut and h5wt, h4wt and h5wt, h4mut and h5mut. This strategy was chosen to take into account the possibility that both the h4 and h5 mutations might be responsible for the inactivation of T-antigen. After primer extension and amplification in bacteria, the resulting DNA molecules were transfected into permissive CV-1 cells. The DNA from Hirt extracts was then linearized with *KpnI* and digested with *DpnI* in order to degrade the input DNA which had not replicated in CV-1 cells. The results of Southern blot analysis are shown in Figure 4. The 7.7-kb pSVTneoZ plasmid, used as a positive control, did replicate (lane A). As expected, use of the h4mut and h5mut oligonucleotide pair combination did not lead to replication of the 6.6-kb pTSVHB plasmid DNA (lane E). In contrast, rescue occurred with h4wt plus h5wt (lane D) indicating that, indeed, at least one of the 2 mutations was responsible for the T-antigen defect. That it is

solely the h4 mutation which is involved is demonstrated by the efficient replication of pTSVHB observed following complementation with the h4wt plus h5mut combination (lane B). This is confirmed by the fact that the h5wt oligonucleotide, in conjunction with h4mut, was inefficient (lane C).

Transforming activity of SV₄₀ DNA from HBL-100 cells

Since we had identified multiple mutations throughout the early region of the SV₄₀ DNA cloned from HBL-100 cells, it was of interest to determine whether any of them might have affected its transforming activity in rodent and human cells. A preliminary experiment was carried out by transfecting recipient human ICIG-7 cells with high-molecular-weight genomic DNA from HBL-100 cells. Three foci were obtained, of which one led to the establishment of a permanent cell line harbouring integrated SV₄₀ DNA, expressing SV₄₀ T-antigen in 100% of the cells and capable of growth in soft agar (data not shown). In order to extend this result and obtain quantitative data, transfection experiments were performed using λ EHBBg, pSVHB1 and pSVHB2 DNAs, in comparison with the origin-minus pLASwt DNA. The latter was chosen as a control in order to take into account the inability of the former DNAs to replicate in the recipient semi-permissive human cells. As shown in Table I, λ EHBBg DNA was able to induce the formation of foci in both mouse 3T3 and human ICIG-7 cells. It was also able to induce the ICIG-7 cells to form colonies in agar. In 2 independent experiments (3 and 4), transforming efficiency was not significantly different from that obtained with control pLASwt DNA. While pSVHB1, which contains the complete SV₄₀ early region, is also transforming, pSVHB2, which harbours a truncated early region, is without effect. Several clones picked at random from mouse 3T3 or human ICIG-7 cells transformed either in liquid medium or in soft agar were all 100% SV₄₀ T-antigen-positive as determined by immunofluorescence. Moreover, several of the transformed ICIG-7 clones could be established as permanent cell lines after a short crisis period (3–4 weeks). Taken together, these results clearly show that the complete early region which has been subcloned in pSVHB1 (Fig. 1), despite multiple mutations, is fully functional for cell transformation.

FIGURE 4 – Marker rescue with synthetic oligonucleotides. Southern blot analysis of *KpnI*- and *DpnI*-digested DNA extracted from CV-1 cells after transfection with plasmid pTSVHB complemented with the following pairs of oligonucleotides: h4wt + h5mut (lane B), h4mut + h5wt (lane C), h4wt + h5wt (lane D), h4mut + h5mut (lane E). Lane A: *KpnI*- and *DpnI*-digested DNA extracted from CV-1 cells after transfection with pSVT neo Z, used as a positive control. Lane F: *EcoRI*-cleaved pTSVHB DNA, used as a marker.

TABLE I – TRANSFORMATION OF MOUSE 3T3-VILL AND HUMAN ICIG-7 CELLS BY CLONED SV₄₀ DNA

Experiment number	Recipient cells	Transfecting DNA ¹	Number of foci ²	Number of agar colonies ²
1	3T3-Vill	λ EHBBg	42	ND ³
2	ICIG-7	λ EHBBg	13	15
3	ICIG-7	pLASwt	28	18
		λ EHBBg	41	23
4	ICIG-7	pLASwt	ND	10
		λ EHBBg	ND	6
5	ICIG-7	pSVHB1	ND	12
		pSVHB2	ND	0

¹In each experiment, control cultures transfected with calf thymus DNA alone never gave rise to foci or agar colonies. ²Per 4×10^6 cells. ³ND = not done.

DISCUSSION

HBL-100 cells obtained from 4 different sources express SV₄₀-specific T-antigen and harbour SV₄₀ DNA integrated in tandem at a unique site (Caron de Fromentel *et al.*, 1985). In the present work we have molecularly cloned the complete viral sequences flanked on both sides by cellular sequences. Subcloning of this DNA allowed us to isolate 2 recombinant plasmids harbouring either a complete SV₄₀ genome (pSVHB1) or an incomplete viral genome with a truncated early region (pSVHB2). Structural analysis of the latter showed that it could code only for a truncated protein of about 34 kDa, but no evidence of expression of such a protein was obtained by immunoprecipitation (data not shown). We therefore concentrated on a detailed study of the pSVHB1 early region capable of expressing the 94-kDa protein previously demonstrated in HBL-100 cells and shown to be defective for viral DNA replication (Caron de Fromentel *et al.*, 1985). In order to locate the mutation(s) involved in this defect, we determined the sequence of pSVHB1 early region. Taking as a reference SV₄₀ wild-type strain 776, the analysis of this sequence revealed the presence of 13 alterations (11 nucleotide substitutions, one 9-bp insertion, one 6-bp deletion). Among these, the point mutation designated h4, at position 3199 and predicting a change at amino-acid 54C of arginine to isoleucine, was the only one responsible for the deficiency of T-antigen, as demonstrated by marker rescue experiments.

By comparison with other SV₄₀ mutants previously mapped in the early region (Fig. 2), the h4 mutation is in the proximity of a cluster of DNA lesions shown to be defective in a function essential to the replication of viral DNA. Moreover, mutant C11b (Manos and Gluzman, 1984) which has an amino-acid change 9 codons downstream from h4, at position 549, is only partially affected in its replication. It appears, therefore, that the novel h4 mutation further delimits one of the domains involved in SV₄₀ DNA replication. No mutations in the coding sequence of large T-antigen have been detected upstream of h4, which is consistent with our previous observations that T-antigen from HBL-100 cells has an intranuclear localization and is capable of binding to the SV₄₀ origin of replication (Caron de Fromentel *et al.*, 1985).

A second issue addressed in the present work concerned the transforming activity of the SV₄₀ genome harboured by HBL-100 cells. That such an activity is preserved has been clearly shown by transfection experiments using either HBL-100 genomic DNA or cloned SV₄₀ DNA sequences. Furthermore, we have shown that this activity is due to the complete early region contained in pSVHB1, despite the various mutations identified. The fact that several of the transformed cell clones that we have isolated were capable of indefinite growth in culture is consistent with our previous hypothesis (Caron de Fromentel *et al.*

al., 1985) that the HBL-100 endogenous SV₄₀ DNA may have been responsible for immortalization of these cells.

Genetic evidence is presently available (Gish and Botchan, 1987) indicating that the lytic replication and the transforming capacity of SV₄₀ are separable by mutation of the viral T-antigen gene. In addition, since human cells are semi-permissive for SV₄₀ replication, it has been suggested that the continued growth of SV₄₀-transformed human cells imposes selective pressure against the replicative function and in favour of the transforming activity of the viral T-antigen gene. These considerations may explain the emergence of the HBL-100 cell

line, the progenitor cells of which—as already suggested (Caron de Fromentel *et al.*, 1985)—might have been infected in the mammary gland of the milk donor by an SV₄₀ virus originally contaminating a polio virus vaccine.

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