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Molecular cloning of integrated simian sarcoma virus: Genome organization of infectious DNA clones

(recombinant DNA/primate retrovirus/restriction enzyme and R-loop analysis/transformation)

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ABSTRACT **The integrated form of simian sarcoma virus (SSV) was molecularly cloned in the Charon 16A strain of bacteriophage λ. In transfection analysis, the recombinant viral DNAs demonstrated the ability to transform cells in tissue culture at high efficiency. Such transformants possessed typical SSV morphology, expressed simian sarcoma associated virus (SSAV) *gag* gene products in the absence of virus release, and released SSV after superinfection with a type C helper virus. A physical map of the 5.8-kilobase-pair (kbp) recombinant viral DNA clone, deduced from restriction endonuclease analysis, revealed a 5.1-kbp SSV genome containing 0.55-kbp-long terminal repeats flanked by 0.45 and 0.25 kbp of contiguous host cell sequences. By R-loop analysis, the viral DNA molecule contained two regions of homology to SSAV, separated by a 1.0-kbp nonhomologous region. This SSV-specific sequence was shown to be uniquely represented within the normal cellular DNA of diverse mammalian species, including human. Our results demonstrate that this primate transforming retrovirus arose in nature by recombination of a type C helper virus and a host cellular gene.**

Transforming retroviruses have been isolated from a number of avian and mammalian species. These viruses appear to have arisen in nature by recombination between a type C helper virus and host cellular information. The latter has been shown to be essential for viral transforming functions (for recent reviews, see refs. 1 and 2). Thus, accumulating evidence indicates that transforming retroviruses may provide an important means of studying cellular genes involved in malignant transformation. The recent application of recombinant DNA techniques to the study of retroviruses has made it possible to isolate and to amplify the genetic information of such viruses for detailed biological and molecular analyses.

The only known transforming retrovirus of primate origin was isolated from a naturally occurring tumor of a woolly monkey (3). This virus, designated simian sarcoma virus (SSV), has been difficult to characterize because it is defective for replication (4), and SSV stocks contain a high excess of an associated type C RNA helper virus, SSAV (4, 5). In an effort to investigate the molecular organization and transforming functions of the SSV genome, we undertook the cloning of SSV proviral DNA from its integrated state within SSV-transformed nonproducer cells.

MATERIALS AND METHODS

Cells and Viruses. Continuous NIH/3T3 (6) and NRK (7) cell lines have been described. Human embryo lung and woolly monkey skin fibroblasts were established in tissue culture from explants. The isolation of clonal NRK cell lines nonproductively transformed by different SSV variants has been reported (8). One such line, which codes for expression of SSAV *gag* gene proteins p12 and p30, was initially referred to as WSV clone 11 (8). This line is designated SSV-11 NRK in the present study. Clonal isolates of simian sarcoma associated virus (SSAV) (4) and mouse amphotropic virus (AP 129) (9) were also utilized.

Enzymes. Avian myeloblastosis virus DNA polymerase was obtained from the Research Resources Area (Division of Cancer Cause and Prevention, National Cancer Institute). Restriction endonucleases were obtained from New England BioLabs. T4 ligase was purchased from Bethesda Research Laboratories (Rockville, MD). T4 polynucleotide kinase and bacterial alkaline phosphatase came from P-L Biochemicals. Reaction conditions were as recommended by the supplier.

Electrophoresis and Molecular Hybridization. Endonuclease-treated DNA was electrophoresed in 0.6–1.2% agarose gels and subjected to Southern blotting analysis as described (10). Fragments containing SSV DNA were identified by hybridization to ^{32}P-labeled DNA probes. SSAV cDNA was prepared by reverse transcription of poly(A)-selected 35S SSAV RNA primed with calf thymus DNA oligonucleotides as described (11). Purified DNA fragments were labeled by the nick-translation method (12). Stringent (13) and relaxed (14) hybridization conditions were used with SSAV and Moloney murine sarcoma virus (MuSV) DNA probes, respectively. DNA fragments detected by molecular hybridization were visualized by autoradiography.

Molecular Cloning. The Charon 16A strain of λ phage was propagated in *Escherichia coli* K-12 DP50supF (15). DNA was purified from CsCl-banded phage as described (16). High molecular weight DNA was extracted from SSV-11 NRK cells by the method of Blin and Stafford (17). *Eco*RI fragments containing SSV DNA were partially purified by preparative agarose gel electrophoresis. *Eco*RI-cleaved vector and partially purified SSV DNA were mixed with T4 ligase and packaged *in vitro* into phage particles as described (18). *In vitro*-packaged recombinant DNA demonstrated 3.5×10^6 plaque-forming units/μg of DNA as compared to 1×10^3 for *Eco*RI-cleaved and 2×10^7 for untreated Charon 16A DNA. Plaques containing SSV DNA were identified by the method of Benton and Davis (19) with SSAV cDNA as probe. All work involving recombinant phage was performed in a P2 containment facility in accordance with National Institutes of Health guidelines for recombinant DNA research.

Electron Microscopy. For R-loop analysis (20), λ–SSV-11 Cl1 DNA (3 μg/ml) was incubated with SSAV 70S RNA (6 μg/ml) for 16 hr at 52°C in hybridization buffer [70% formamide/0.1 M *N*-tris(hydroxymethyl)methylglycine (Tricine)·NaOH, pH 8.0/0.5 M NaCl/10 mM EDTA]. The sample was diluted 1:10

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Abbreviations: SSV, simian sarcoma virus; SSAV, simian sarcoma associated virus; MuSV, murine sarcoma virus; LTR, long terminal repeat; kbp; kilobase pair(s).

in hybridization buffer containing cytochrome at 50 μg/ml and an aliquot was spread onto a hypophase of H_2O. The nucleic acids were transferred to Parlodion-coated grids, stained with 0.1 mM uranyl acetate in 90% ethanol, and shadowed with platinum/palladium. Nucleic acid contour lengths were measured with the aid of a Tektronix computerized graphic system. The φX174 DNA and its replicative form were used as molecular weight standards.

Radioimmunoassays. The M_r 12,000 (p12) and 30,000 (p30) structural proteins of SSAV were isolated as described (8). Each was labeled with ^{125}I at high specific activity (10 μCi/μg; 1 Ci = 3.7 × 10^{10} becquerels) by the chloramine-T method of Greenwood *et al.* (21). Competition radioimmunoassays were performed as reported (22).

DNA Transfection. Transfection of NIH/3T3 cells with molecularly cloned or cellular DNA was performed by the calcium phosphate precipitation technique (23) as modified by Wigler *et al.* (24). Briefly, 1 ml of 0.25 M $CaCl_2$ containing varying amounts of test DNA and 50 μg of salmon sperm DNA as carrier was mixed with an equal volume of a 50 mM Hepes, pH 7.1/280 mM NaCl/1.5 mM sodium phosphate. The calcium phosphate precipitate was added to a 10-cm Petri dish, in which 1–2 × 10^5 NIH/3T3 cells had been plated 24 hr earlier. After overnight incubation, the supernatant was removed, and 10 ml of Dulbecco's modified Eagle's medium supplemented with 5% calf serum was added. Transformed foci were scored at 14–21 days.

For analysis of SSV rescue, individual transformed foci induced at limiting DNA concentration were picked by the cloning cylinder technique, grown to mass culture, and superinfected with mouse amphotropic virus. Tissue culture fluids were assayed 2–3 weeks later for focus-forming activity on NIH/3T3 cells (25).

RESULTS

Selection of Restriction Endonuclease Suitable for Molecular Cloning of Integrated SSV. In order to achieve molecular cloning of infectious SSV provirus in its integrated form, we attempted to find a restriction enzyme compatible with available vectors that would not cleave SSV proviral DNA. DNA prepared from SSV-11 NRK nonproducer cells was treated with restriction enzymes including *Bam*HI, *Hin*dIII, *Sal* I, and *Eco*RI and subjected to Southern blotting analysis using SSAV cDNA as a probe. Cleavage by *Eco*RI resulted in a single 5.8-kilobase-pair (kbp) DNA band; each of the other enzymes tested yielded more than one band (data not shown).

We next compared the transforming activities of *Eco*RI-cleaved and untreated SSV-11 NRK DNA by transfection analysis. Their focus-forming activities on NIH/3T3 cells were found to be similar and ranged from 3 to 10 focus-forming units/100 μg of cellular DNA. Moreover, when transformed foci induced by *Eco*RI-cleaved SSV-11 NRK DNA were superinfected with mouse amphotropic type C virus, transforming virus that induced foci with morphologic features characteristic of SSV was rescued. All of these results suggested that *Eco*RI was suitable for cloning intact SSV from SSV-11 NRK cellular DNA.

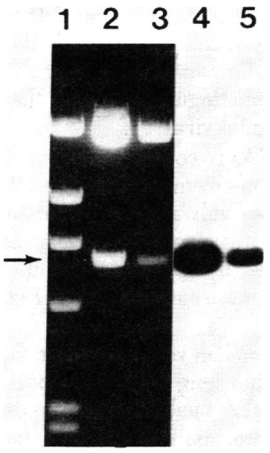

FIG. 1. Electrophoretic analysis of λ–SSV recombinant DNA clones. DNAs extracted from plaque-purified λ phages, λ–SSV-11 Cl1 and Cl2, were digested with *Eco*RI, electrophoresed on agarose gels, and stained with ethidium bromide (lanes 2 and 3, respectively). Fragments containing SSAV nucleotide sequences were identified by Southern blotting analysis using SSAV cDNA as a probe (lanes 4 and 5, respectively). Fragments of *Hin*dIII–digested λ DNA served as size standards (lane 1). Arrowhead, 5.8 kbp.

Molecular Cloning of the Integrated SSV Genome. *Eco*RI-cleaved SSV-11 NRK DNA was subjected to agarose gel electrophoresis in an effort to enrich for SSV proviral sequences. The 5.8-kbp DNA fraction was ligated to *Eco*RI-cleaved DNA purified from the Charon 16A strain of λ phage (15), packaged *in vitro* into phage particles as described (18), and plated onto *E. coli* K-12 DP50supF. The resulting plaques were tested for reactivity with SSAV cDNA by the method of Benton and Davis (19). Of 1.7 × 10^5 plaques analyzed, 2 were scored as positive. Each was plaque-purified twice prior to further analysis. DNA extracted from the purified phage clones, designated λ–SSV-11 Cl1 and Cl2, was treated with *Eco*RI and subjected to Southern blotting analysis. In each case, *Eco*RI-cleavage yielded a 5.8-kbp insert which was readily detected by hybridization with SSAV cDNA (Fig. 1).

Biological Activity of Cloned SSV DNA. To determine whether the recombinant DNA clones possessed biological activity, they were analyzed by transfection on NIH/3T3 cells. λ–SSV-11 Cl1 and Cl2 DNAs each demonstrated high-titered focus-forming activity that was not significantly altered by *Eco*RI cleavage (Table 1). Focus formation was a linear function of amount of DNA added, indicating that a single DNA molecule was able to induce a transformed focus. When individual transformed foci were selected by the cloning cylinder technique, grown to mass culture, and superinfected with amphotropic mouse type C helper virus, focus-forming activity characteristic of SSV was rescued from each transformant tested. Previous studies have shown that SSV-11 NRK cells express SSAV *gag* gene encoded p12 and p30 (8). We assayed transformants induced by SSV recombinant DNA clones for expression of these

Table 1. Biologic activity of λ–SSV-11 recombinant DNA clones

Recombinant DNA	*Eco*RI cleaved	Foci/plate,* no. DNA added 1.0	0.1	0.01	Specific infectivity†	Transformants with rescuable SSV,‡ no. rescuable/no. tested
λ–SSV-11 Cl1	No	>150	42, 60	3, 4	$10^{4.2}$	4/4
	Yes	>150	57, 63	4, 5	$10^{4.3}$	4/4
λ–SSV-11 Cl2	No	70	8, 8	1, 0	$10^{3.4}$	4/4
	Yes	85	12, 9	1, 1	$10^{3.6}$	4/4

* DNA transfection was performed with 50 μg of salmon sperm DNA as carrier. Focus formation on NIH/3T3 cells was scored at 14–21 days. DNA added is shown as μg/plate.

† Shown as focus-forming units/pmol of viral DNA.

‡ Individual transformed foci were isolated at limiting DNA concentration, grown to mass culture, and superinfected with a mouse amphotropic virus. At 2–3 weeks, tissue culture fluids were assayed for focus formation on NIH/3T3 cells.

proteins and found that they expressed SSAV p12 and p30 at levels ranging from 30 to 120 ng/mg of cell protein (data not shown). Thus, the phenotype of transformants induced by the SSV recombinant DNA clones was indistinguishable from that of transformants induced by the parental virus.

Physical Map of λ–SSV-11 Cl1 DNA. To construct a physical map of λ–SSV-11 Cl1 DNA, restriction enzyme cleavage products were electrophoresed on agarose gels and visualized by ethidium bromide staining. The relative location of cleavage sites on a linear map was determined by appropriate double digestions. The results of these studies are summarized in Fig. 2.

Proviral DNAs of avian and mammalian retroviruses so far examined have been shown to contain long terminal repeats (LTR)s (27–30). We attempted to localize analogous regions on the λ–SSV-11 Cl1 physical map by determining whether a specific constellation of restriction enzyme sites was repeated within the molecule. *Pst* I and *Kpn* I cleaved the molecule in identical locations with respect to two *Sac* I sites located near either terminus at approximately 0.8 and 5.5 kbp on the restriction map. It seemed possible that these regions, each approximately 0.5 kbp long, might correspond to the LTRs of SSV.

In order to establish more firmly the presence of LTRs and define their length, we constructed a detailed restriction map of the terminal 1.3- and 1.0- kbp *Xba* I fragments of λ–SSV-11 Cl1 DNA. *Hin*fI and *Alu* I were chosen for this purpose because the sites at which they cleave occur frequently in DNA. An identical constellation of two *Hin*fI and four *Alu* I sites was observed in each fragment within the regions defined by *Pst* I and *Kpn* I sites. Several unique *Hin*fI and *Alu* I sites were detected outside of these domains. These results demonstrated that terminal redundancies were present in SSV-11 DNA and defined their length as 0.55 ± 0.02 kbp. From the location of the two LTRs within λ–SSV-11 Cl1 DNA, it was possible to establish the length of SSV genomic DNA as 5.1 kbp and the presence of flanking NRK cellular DNA sequences extending 0.45 and 0.25 kbp to the left and right ends, respectively, of the proviral DNA.

Orientation of λ–SSV-11 Cl1 DNA with Respect to SSV RNA. It is known that the viral *gag* gene resides within the 5′ region of the type C viral RNA genome (for review, see ref. 31). The fact that SSV-11 codes for SSAV *gag* gene products made it possible to devise a means of assigning these coding sequences to a specific region of the λ–SSV-11 Cl1 DNA physical map. We took advantage of knowledge that mammalian type C viruses are evolutionarily related and of the availability of a molecular clone of Moloney MuSV DNA (32). Moloney MuSV DNA subclones composed of murine leukemia virus LTR or *gag* gene sequences were nick-translated and hybridized to Southern blots of *Xba* I-cleaved λ–SSV-11 Cl1 DNA. The four *Xba* I fragments (Fig. 3, lane 2) were ordered 1.3, 1.6, 1.9, and 1.0 kbp from left to right on the λ–SSV-11 Cl1 physical map (Fig. 2). As shown in lane 3 of Fig. 3, the LTR subclone of Moloney MuSV DNA detected only the 1.3- and 1.0-kbp *Xba* I fragments which were shown above (Fig. 2) to contain the SSV LTRs. In contrast, the Moloney MuSV *gag* gene probe hybridized with the 1 3- and 1.6- but not the 1.9- or 1.0-kbp fragments (Fig. 3, lane 4). These results localized SSV *gag* gene-related information at the left end of the physical map of λ–SSV-11 Cl1 DNA and thus oriented this end as 5′ with respect to SSV genomic RNA.

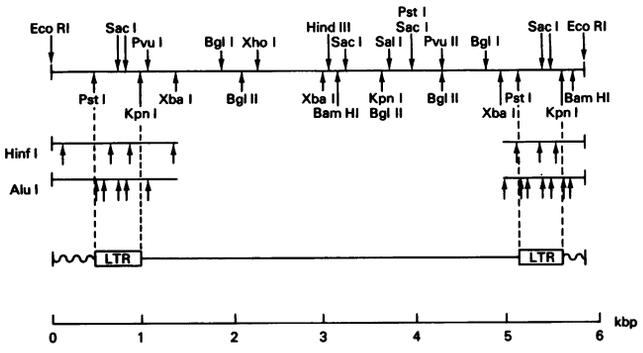

FIG. 2. Restriction map of λ–SSV-11 Cl1 DNA. Sites of restriction enzyme cleavage within the DNA insert were determined by double digestion analysis. The sites of *Hin*fI and *Alu* I cleavage within the terminal *Xba* I DNA fragments were determined by using the partial digestion method of Smith and Birnstiel (26). Wavy lines, NRK cellular flanking sequences; LTR, long terminal repeat.

Detection and Localization of SSV Nucleotide Sequences Not Found in SSAV. The transforming retroviruses studied so far have been shown to contain nucleotide sequences unrelated to those of a type C helper virus in addition to helper virus-related information (for recent reviews, see refs. 1 and 2). In an effort to detect and to localize such sequences in λ–SSV-11 Cl1 DNA, we performed R-loop analysis of this clone with SSAV genomic RNA. A representative of 30 R-loop structures analyzed is shown in Fig. 4. Two regions of DNA·RNA homology, 3.2 ± 0.2 and 0.7 ± 0.01 kbp, were detected. Within the larger region of homology, two loops of single-stranded RNA representing SSAV sequences deleted from the SSV genome were observed. Of particular note was the finding that the two regions of DNA·RNA homology were separated by 1.0 ± 0.1 kbp of λ–SSV-11 Cl1 DNA that lacked homology with SSAV RNA. These results indicated that λ–SSV-11 Cl1 contained a contiguous 1.0-kbp region that was unrelated to helper virus and was located within the transforming virus genome.

In order to orient the R-loop structure with respect to SSV genomic RNA and the λ–SSV-11 Cl1 DNA physical map, we took advantage of the fact that SSV codes for a *gag* gene product containing SSAV p12 and p30. Only the larger region of DNA·RNA homology possessed sufficient coding capacity for this gene product. Thus, the 3.2- and 0.7-kbp regions of DNA·RNA homology must be located 5′ and 3′, respectively, on SSV genomic RNA.

As an independent approach toward localization of SSAV unrelated sequences within λ–SSV-11 Cl1 DNA, we utilized the Southern blotting technique. From the results of R-loop analysis, the region located at approximately 4.0–5.0 kbp on the λ–SSV-11 Cl1 DNA physical map (Fig. 2) should contain information unrelated to SSAV. To test this possibility, a 1.6-kbp *Sac* I DNA fragment located 4.0–5.6 kbp on the SSV-11 Cl1 DNA physical map was purified. After cleavage with *Bgl* II, *Bgl* I, or *Xba* I, the resulting fragments were subjected to Southern blotting analysis and hybridization with a nick-translated 8.8-kbp SSAV DNA that had been cloned from the circular form of the unintegrated provirus (P. R. Anderson, personal communication).

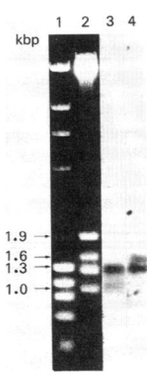

FIG. 3. Orientation of λ–SSV-11 Cl1 DNA with respect to SSV RNA. λ–SSV-11 Cl1 DNA was treated sequentially with *Eco*RI and *Xba* I. After agarose gel electrophoresis, the DNA cleavage products were visualized by staining with ethidium bromide (lane 2.) The same DNA fragments were also analyzed by Southern blotting and hybridization with nick-translated DNA subclones of Moloney MuSV composed of its LTR (lane 3) and *gag* gene (lane 4) sequences. These probes were derived from 0.6- to 1.2- and 2.4- to 2.8-kbp regions, respectively, on the restriction map of the *Hin*dIII-permuted Moloney MuSV DNA genome (32). *Hin*dIII-digested λ and *Hae* III-digested φX174 replicative form DNAs served as size standards (lane 1).

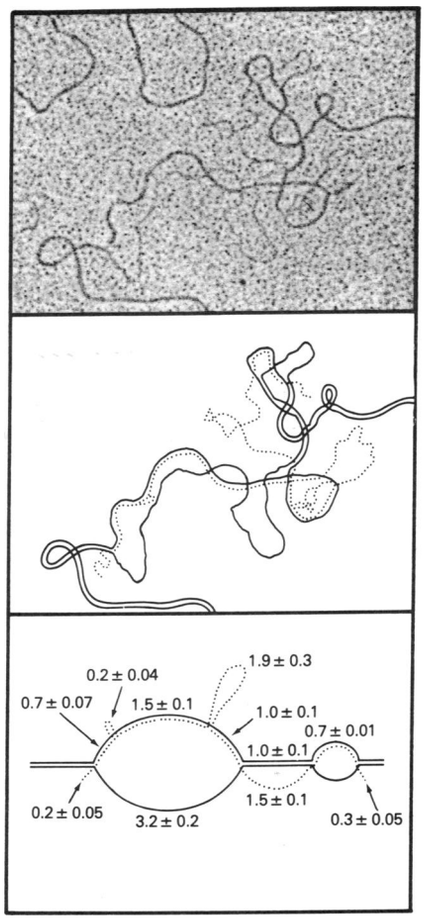

FIG. 4. R-loop analysis of λ–SSV-11 Cl1 DNA and SSAV RNA. Solid line, λ–SSV-11 Cl1 DNA; dotted line, SSAV RNA. The contour lengths indicated are expressed in kb and represent the mean ± SD obtained from analysis of 30 separate molecules.

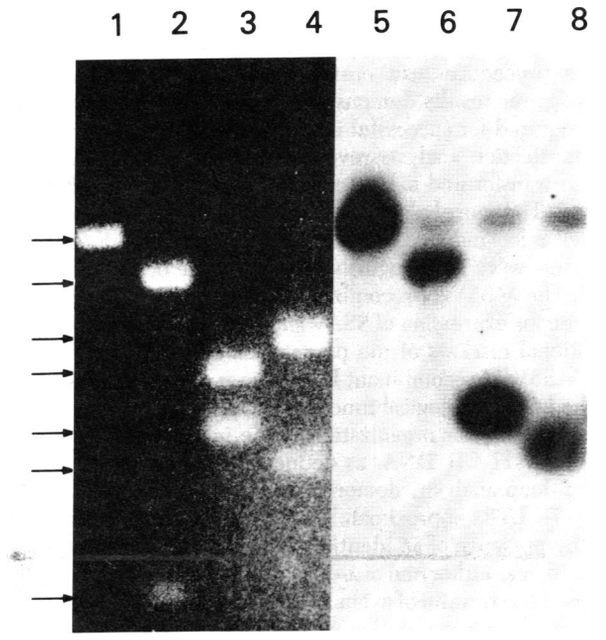

FIG. 5. Localization of nucleotide sequences unrelated to SSAV on the restriction map of λ–SSV-11 Cl1 DNA. A DNA fragment located at 4.0–5.6 kbp on the λ–SSV-11 Cl1 restriction map was purified by agarose gel electrophoresis after *Sac* I cleavage of λ–SSV-11 Cl1 DNA. The purified 1.6-kbp DNA fragment (lane 1) as well as DNA products resulting from cleavage with *Bgl* II (lane 2), *Bgl* I (lane 3), and *Xba* I (lane 4) were visualized by ethidium bromide staining. DNA fragments containing SSAV-related nucleotide sequences were identified by Southern blotting and hybridization with nick-translated, molecularly cloned SSAV genomic DNA as probe (lanes 5–8, respectively). Fragments of *Hin*dIII-digested λ and *Hae* III-digested φX174 replicative form DNAs served as size standards (arrows from top to bottom): 1.6, 1.3, 1.05, 0.95, 0.72, 0.61, and 0.29 kbp.

The purified 1.6-kbp *Sac* I DNA fragment (Fig. 5, lane 1) was cleaved once by each enzyme utilized. DNA fragments of 1.3 and 0.29 kbp (lane 2), 0.95 and 0.72 kbp (lane 3), and 1.05 and 0.61 kbp (lane 4) resulted from cleavage with *Bgl* II, *Bgl* I, and *Xba* I, respectively. The SSAV DNA probe hybridized to the 1.6-kbp *Sac* I DNA fragment (lane 5). However, only the 1.3-kbp *Bgl* II (lane 6), 0.72-kbp *Bgl* I (lane 7), and 0.61-kbp *Xba* I (lane 8) cleavage products were detected by this probe. The remaining fragments lacked detectable homology to SSAV. Thus, the region encompassed by *Sac* I and *Xba* I sites and located at 4.0–5.05 kbp on the physical map of λ–SSV-11 Cl1 DNA was composed of SSV nucleotide sequences not represented within the SSAV genome.

Cellular Origin of the SSV-Specific Sequence. The molecular organization of SSV proviral DNA closely resembles that of Moloney MuSV (32, 33), leading us to consider whether SSV-specific sequences, by analogy with those of MuSV, were cellular in origin. To test this possibility, we utilized the DNA fragment located at 4.0–5.05 kbp on the physical map of λ–SSV-11 Cl1 DNA as a probe to search for sequence homology with mammalian cellular DNAs. Southern blotting analysis of *Eco* RI-cleaved SSV-11 NRK cellular DNA revealed the 5.8-kbp DNA fragment containing integrated SSV DNA (Fig. 6). In addition, an SSV-related 15-kbp band, also present in rat cell DNA (lane 2), was readily visualized. The DNA probe also detected a single 23-kbp band in DNAs of both normal woolly monkey and human cells (lanes 3 and 4). These findings demonstrate that the region of the SSV genome that lacks homology with SSAV is related to cellular DNA sequences present in single or low copy number within the mammalian genome.

DISCUSSION

The recent application of recombinant DNA techniques to the study of retroviruses has made these viruses much more amenable to detailed biochemical analysis. Our strategy for molecular cloning of the primate transforming retrovirus SSV involved isolation of a DNA fragment containing intact, linear SSV DNA and host flanking sequences from cells nonproductively transformed by a clonal SSV variant. This approach required first the selection of a restriction enzyme, *Eco*RI, that did not cleave within the integrated viral genome. We recovered one SSV recombinant DNA phage per 10^5 plaque-forming units

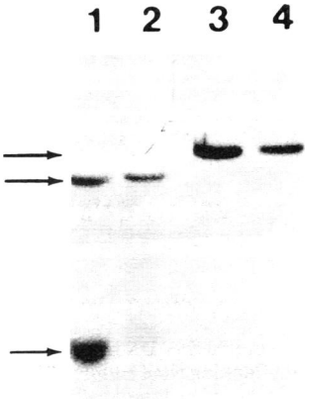

FIG. 6. Detection of homology between normal cellular DNA and SSV nucleotide sequences not found in SSAV. Cellular DNAs were treated with *Eco*RI, fractionated by agarose gel electrophoresis, and subjected to Southern blotting analysis using, as a hybridization probe, the SSV-specific DNA fragment derived from 4.0–5.0 kbp on the restriction map of λ–SSV-11 Cl1 DNA. Cellular DNAs included those of SSV-11 NRK (lane 1), uninfected NRK (lane 2), woolly monkey fibroblasts (lane 3), and human embryo lung fibroblasts (lane 4). Size markers (arrows from top to bottom): 23.0, 15.0, and 5.8 kbp.

after enrichment for viral sequences by a single cycle of preparative agarose gel electrophoresis. Whereas additional purification procedures can enrich further for a particular gene (33–35), our results demonstrate that extensive purification is not required for successful isolation of single copy genes.

Transfection analysis revealed that the recombinant DNA clones transformed mouse cells in tissue culture at a high efficiency. The morphologic characteristics of DNA transformants as well as foci induced by sarcoma virus rescued from such transformants were indistinguishable from those of foci induced by SSV. The λ–SSV-11 recombinant DNA clones were also shown to code for expression of SSAV *gag* gene products p12 and p30, additional markers of the parental SSV-11 genome (8). Thus, the λ–SSV-11 recombinant DNA clones appeared to possess all of the known biological functions of SSV.

The molecular organization of the integrated SSV provirus in λ–SSV-11 Cl1 DNA, as deduced from restriction mapping and R-loop analysis, demonstrated several important features (Fig. 7). LTRs approximately 0.55 kbp in length were defined by the presence of an identical constellation of restriction sites located near either end of λ–SSV-11 Cl1 DNA. Analogous structures at the termini of avian and murine retroviruses have been shown to contain putative regulatory signals for initiation and termination of transcription (36–38). Nucleotide sequence analysis of retroviral LTRs has also indicated their structural similarities with transposable elements, suggesting that the LTR may play an important role in proviral DNA integration into the cellular genome.

R-loop analysis helped to explain the basis for the replication-defective nature of SSV. From knowledge of the type C viral gene order as 5′ *gag-pol-env* 3′ (31), the 1.5-kb deletion of SSAV sequences toward the 3′ end of the viral RNA was likely to lie within the SSAV *env* gene. Of the two SSAV deletion loops detected within the major 3.2-kbp region of SSV/SSAV homology, the larger likely corresponded to the region of the SSAV *pol* gene. Although the smaller deletion (0.2 kb) appeared to be located at or near the beginning of the viral *gag* gene, this deletion did not impair the ability of our SSV recombinant DNA clones to synthesize SSAV *gag* gene products. Precise localization of SSAV coding sequences deleted from SSV will require detailed nucleotide sequence comparison of molecularly cloned SSAV with our SSV recombinant DNA clones.

The 5.1-kbp SSV DNA genome was shown to be composed of SSAV homologous regions, 3.2 and 0.7 kbp, respectively, at 5′ and 3′ ends of the molecule connected by a continuous 1.05-kbp region of SSAV nonhomologous information. Nucleotide sequences related to the SSV-specific region were detected at single copy number within normal cellular DNAs of several mammalian species. These findings strongly argue that SSV arose by recombination of SSAV with a well-conserved cellular gene. Because the cloned SSV-specific sequence readily detected related information in human cellular DNA, it should be possible to determine whether altered or enhanced expression of SSV related sequences is involved in naturally occurring human malignancies.

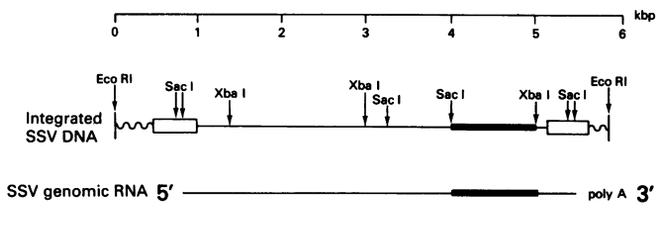

FIG. 7. Molecular organization of integrated SSV DNA in SSV-11 NRK cellular DNA. Wavy lines represent flanking NRK cellular DNA sequences. Open boxes represent LTRs. Darkened rectangle represents SSV nucleotide sequences not found in SSAV.

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