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Molecular cloning and comparative analyses of the genomes of simian sarcoma virus and its associated helper virus

(recombinant DNA/long terminal repeats)

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ABSTRACT **Closed circular viral DNA of simian sarcoma virus (SSV) and simian sarcoma-associated virus (SSAV) obtained from acutely infected dog cells was purified on preparative agarose gels, cleaved with *Eco*RI, and cloned in the phage λ vector Charon 21A. The cloned 9-kilobase SSAV genome (B11) has the same restriction map as the bulk of the unintegrated linear SSAV DNA intermediate. Heteroduplex analysis between an SSV clone (λ-C60) and an SSAV clone (λ-B11) showed two substitution loops and one deletion loop. By using detailed restriction enzyme mapping and electron microscopic analysis, we showed that one of the substitution loops corresponds to an inversion of one of the two long terminal repeat units and adjacent cellular sequences in C60. The other substitution loop mapped close to the 3′ long terminal repeat. At least part of this region was shown to contain SSV-specific sequences not shared by SSAV. The 1.9-kilobase deletion mapped at 3.5–5.5 kilobases of the linear SSAV genome, corresponding to most, if not all, of the *pol* gene.**

The simian sarcoma virus (SSV) and its simian sarcoma-associated (helper) virus (SSAV) belong to a unique family of horizontally transmitted primate retroviruses. Other members of this family include various isolates of gibbon ape leukemia viruses (GaLV) (1). These are the only known retroviruses clearly associated with leukemias, lymphomas, and sarcomas in primates (2). SSV/SSAV was first isolated from a spontaneous fibrosarcoma of a pet woolly monkey (3, 4). SSV is tumorigenic when inoculated into other primates (2). The virus can be grown in cultured cells of many species and induces transformation *in vitro*. Like other mammalian sarcoma viruses, SSV is defective and requires a helper virus for replication (4, 5).

SSAV is closely related to GaLV antigenically and in their genomes as measured by molecular hybridization (6–8). It is not endogenous to primates and has no relationship to other primate endogenous viruses, such as the baboon endogenous virus (BaEV) (7, 9). Molecular hybridization and immunological studies suggest that SSAV and GaLV originated from an endogenous virus of rodents (7, 10–12). Because of its low titer, the sarcoma virus genetic structure is not as well defined. Its origin is also not known and may be from either rodent, primate, or other species.

Like the bovine leukemia virus and other groups of primate retroviruses, the molecular biology of the SSV/SSAV and GaLV group of viruses is in an earlier phase of development than the murine, avian, or feline retroviruses. However, we can infer from studies of avian and murine retroviruses that SSAV has a linkage map common with that of all other nondefective viruses: a 5′–3′ order of three genes (*gag*, *pol*, and *env*) that code for a precursor of the core proteins, viral polymerase, and the envelope glycoproteins, respectively (13). Further, like all other defective transforming viruses (13–16), SSV has probably arisen by recombination of SSAV and a subset of cellular DNA sequences of a yet unidentified host, although there is no direct evidence for this.

Retroviruses replicate via a double-stranded DNA intermediate that is incorporated into host cell DNA as a provirus (13). The viral DNA contains long terminal repeat units (LTRs) of 300–1200 base pairs (bp) (17–21). These units are structurally similar to bacterial insertion elements and, like the latter, may regulate expression of adjacent cellular sequences (22). The LTRs contain the binding site for RNA polymerase and signaling sequences for polyadenylylation (21); they are important for viral DNA integration and have also been associated with intragenomic inversions (23) and deletions in cloned DNA (24, 25).

We report here cloning of unintegrated SSV and SSAV genome DNA, isolated from newly infected cells and cleaved at the unique *Eco*RI site. The SSAV clone has two direct LTRs in tandem and a restriction enzyme map identical to that of linear viral DNA except that it is permuted at the *Eco*RI site. The SSV clone contains a 1.9-kilobase (kb) deletion in the *pol* region and a 0.9-kb substitution before the 3′ LTR. At least part of this substitution contains SSV-specific sequences not shared by SSAV.

MATERIALS AND METHODS

Preparation of Extrachromosomal SSV and SSAV DNA. Approximately 10^9 canine thymus cells (A7573) were infected at a multiplicity >10 with SSV/SSAV from the marmoset tumor cell line 71AP1 (3, 4). The cells were harvested 24 hr after infection, and low molecular weight DNA was isolated according to Hirt's procedure (26). The DNA was applied to a 1.0% preparative agarose gel and subjected to electrophoresis at 200 mA for 24 hr. We cut a vertical strip of the gel and transferred its DNA to diazobenzyloxymethyl paper for hybridization to [^{32}P]cDNA prepared from 70S RNA of SSV/SSAV (71AP1). Conditions for hybridization were as described by Wahl *et al.* (27). Three bands were detected by autoradiography. We excised these bands from the gel, dissolved each slice in saturated NaI, and purified the DNA by binding to glass powder (28). The three fractions were analyzed by restriction enzyme digestion and used for ligation to phage λ DNA.

Cloning Circular SSAV and SSV DNA. Charon 21A phage DNA was treated with *Eco*RI (Bethesda Research Laboratories, Rockville, MD), which cuts linear SSAV DNA once. We found that the two faster migrating bands from the preparative gel fractions contained closed circular SSV/SSAV DNA; their gel migration was retarded after digestion with *Eco*RI. We ligated

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Abbreviations: SSV, simian sarcoma virus; SSAV, simian sarcoma-associated (helper) virus; GaLV, gibbon ape leukemia virus; LTR, long terminal repeat; kb, kilobase(s); bp, base pair(s).

the *Eco*RI-digested DNA from each of these two fractions to Charon 21A phage DNA arms (29) and transferred the ligation mixture directly to an *in vitro* packaging reaction (30). Cloned SSV/SSAV DNA was selected by the plaque amplification method (31). We screened all positive plaques by extracting the phage DNA directly from a 2-ml aliquot of phage lysate, digesting it with restriction enzymes, and analyzing it by Southern blot (32) hybridization to SSV/SSAV [^{32}P]cDNA (33). Thus, we were able to classify clones, which were then plaque-purified three times.

Preparation and Analysis of Cloned DNA. We isolated phage from large-scale lysates in 500 ml of NZ-amine broth (29) by the method of Thomas and Davis (34). Restriction endonuclease digestion was performed as recommended by the supplier. Blot hybridization was carried out as described by Southern (32). Heteroduplex formation was carried out in 50% formamide at 25°C after alkali denaturation of cesium-banded phage particles or agarose-purified DNA. Heteroduplex spreading used a hyperphase of 40% formamide and a hypophase of 10% formamide (35). Single- and double-stranded ϕX174 DNA was included on all grids as an internal size marker. Lengths determined are an average of at least 10 molecules and have SD <5%.

All recombinant DNA procedures were carried out under P-2 conditions specified by the National Institutes of Health guidelines for recombinant DNA research.

RESULTS

Molecular Cloning of SSAV and SSV DNA. Linear and closed circular unintegrated SSV/SSAV DNA was obtained from a Hirt supernatant of acutely infected cells. The low molecular weight DNA extracted from the infected cells was subjected to electrophoresis on a preparative agarose gel. We transferred the DNA from a vertical slice of the gel to diazobenzyloxymethyl paper. Hybridization of SSV/SSAV [^{32}P]cDNA to the paper showed three bands. The three corresponding regions were excised from the preparative gel, and the DNA was isolated (Fig. 1). Band A was 9.0 kb long, which corresponds to the size of full-length unintegrated SSAV. *Sal* I and *Xho* I restriction enzyme digestions gave fragments characteristic of linear unintegrated SSAV DNA, as shown on the map in Fig. 1. *Eco*RI digestion of the DNA in band B results in one cut and retards the gel migration of that band so that it comigrates with band A. Some undigested DNA in band B had been nicked during purification from agarose and migrates at 9.0 kb (linear) and above (open circles). Thus, band B represents the closed circular DNA of SSAV. Similarly, DNA isolated from the fastest migrating band, C, was also retarded on agarose gel after *Eco*RI digestion. However, these linear molecules clustered in a heterogeneous set of bands centering at 7.5 kb. Restriction digests of band B DNA show internal fragments that comigrate with fragments from band A and fusion fragments of the 5′ and 3′ ends of the linear molecule, as expected from the map. The analysis of the fragments from band C before cloning is more complex due to the heterogeneity of the DNA molecules.

DNA from bands B and C were ligated separately to *Eco*RI-cleaved Charon 21A DNA. *In vitro* packaging of ligated DNA gave 2.3×10^5 phage per μg of ligated λ phage DNA for λ-B and 8.5×10^4 phage per μg for λ-C. Approximately 20,000 plaques of each were screened. Of 16 positive signals from λ-B, 5 clones were confirmed by restriction enzyme digestion of phage DNA. All 5 λ-B inserts were 9.0 kb long, and a limited restriction enzyme analysis suggested that they represent the SSAV genome. One, λ-B11, was chosen for further characterization. Of 63 positive signals from λ-C, 12 clones were confirmed by *Eco*RI digestion of phage DNA. The insert DNA varied from 6.0 to 7.5 kb. *Hin*dIII digestion of the λ-C phage DNA showed one of these clones, λ-C60, to lack two widely spaced *Hin*dIII sites in λ-B11 (at 4.3 and 7.1 kb of the linear map in Fig. 1), while the other λ-C clones contained at least one of these two sites. We directed our attention to analysis of λ-C60 because it seemed more likely to contain substitution regions.

Heteroduplex Analysis of Clones λ-B11 and λ-C60. To obtain a visual display of the regions of homology and nonhomology between λ-B11 and λ-C60, we disrupted the two recombinant phages and formed heteroduplexes between the DNAs. A typical heteroduplex is shown in Fig. 2*A*. The long phage arms

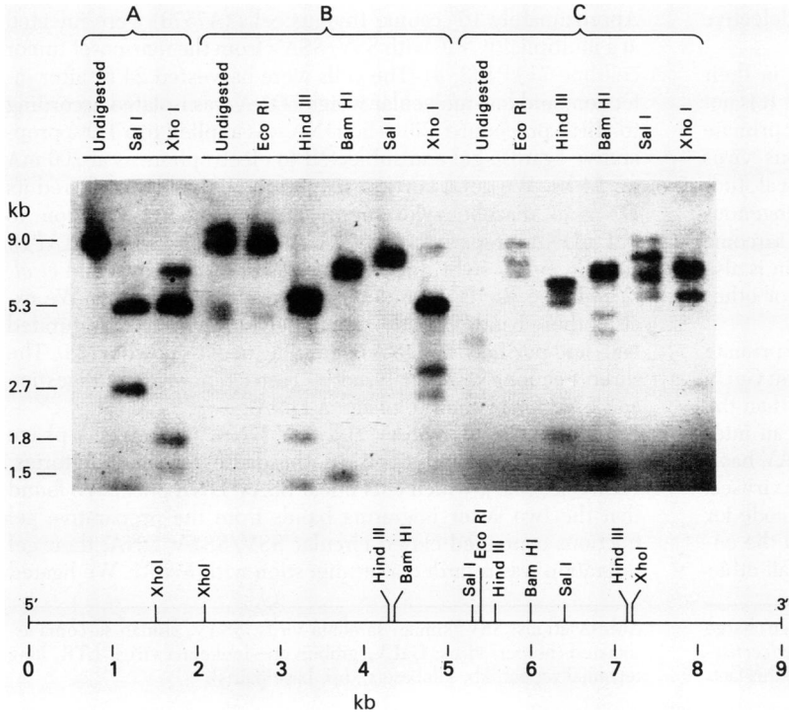


FIG. 1. Restriction enzyme analysis of SSV/SSAV unintegrated DNA preparative gel fractions. DNA was purified from a Hirt fractionation of freshly infected dog cells as described in *Materials and Methods*. Band A: Nine-kilobase linear unintegrated SSAV DNA. *Sal* I and *Xho* I endonucleolytic fragments correspond to those expected from the map as shown. *Sal* I fragments, 5.3 kb, 2.7 kb, and 1.0 kb; *Xho* I fragments, 5.3 kb, 1.8 kb, 1.4 kb, and 0.6 kb (off gel). Band B: Supercoiled DNA from the intermediate migrating band was partly nicked during extraction from agarose to yield open circles (running behind the 9.0-kb front) and linear DNAs (comigrating with the 9.0-kb material). *Eco*RI cleaves most of the DNAs to the linear form. Other restriction fragments correspond to the internal fragments of the linear DNA and 5′–3′ end fused fragments. *Hin*dIII fragments, 6.1 kb (fusion), 1.8 kb, 1.1 kb; *Bam*HI fragments, 7.7 kb (fusion), 1.3 kb. *Sal* I fragments, 8.0 kb (fusion), 1.0 kb (off gel). *Xho* I fragments, 5.3 kb, 3.1 kb (fusion), 0.6 kb (off gel)—the 3.7-kb fragment may have resulted from a subpopulation of molecules lacking the *Xho* I site at 1.4 kb of the map. Band C: Supercoiled DNA representing molecules smaller than the full-length helper virus. These give a heterogeneous pattern on endonucleolytic cleavage.

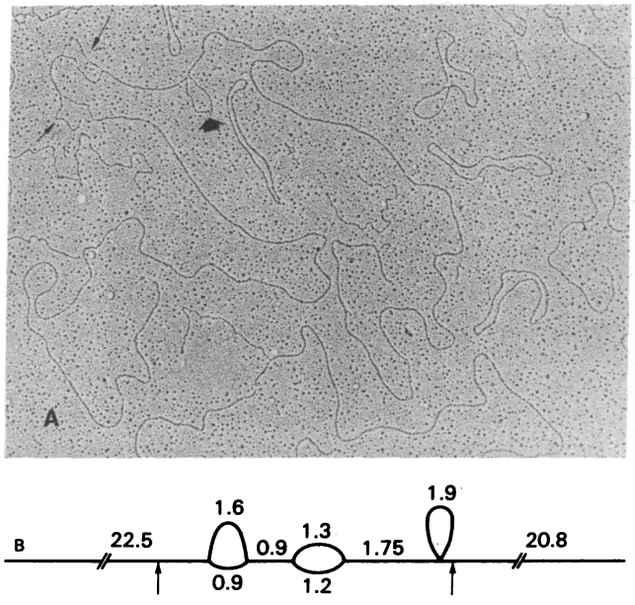


FIG. 2. (*A*) Electron micrograph of heteroduplex between λ-C60 and λ-B11. Note the two substitutions (thin arrows) and the deletion loop (thick arrow) in the midst of the long λ phage arms. (×14,700.) (*B*) Schematic of the micrograph. Lengths in kb are calculated from internal size markers of single- and double-stranded φX174 DNA. Arrows indicate approximate locations of the phage-insert junctions.

flank two substitution loops and one deletion loop with interspersed homologous stretches. The schematic (Fig. 2*B*) shows the approximate sizes of the heteroduplex segments. The junctions of the λ arms and the viral insert cannot be seen, but the measurements of the long phage arms suggest that the first substitution is ≈1.2 kb from one junction. The second substitution loop is separated from the first by 0.9 kb of double-stranded region. The 1.9-kb deletion is very close to the other phage insert junction. We proceeded to characterize the nature of the three regions of difference between λ-B11 and λ-C60.

Restriction Enzyme Mapping of Clones λ-B11 and λ-C60. We determined digestion sites for 12 restriction enzymes on both λ-B11 and λ-C60 (Fig. 3). The sites on λ-B11 are identical to those determined for linear unintegrated SSAV DNA (M. S. Reitz *et al.*, unpublished results) except that B11 is permuted at the single *Eco*RI site. The 5′–3′ orientation of the cloned DNA with respect to the virus genome is as indicated. At least three restriction enzymes have known cleavage sites in the LTR of linear unintegrated SSAV: *Pst* I, *Sst* I, and *Kpn* I (M. S. Reitz *et al.*, unpublished results). Two adjacent 500-bp regions centered at 3.6 kb of the B11 map have identically positioned sites for digestion by *Pst* I, *Sst* I, and *Kpn* I. The open boxes in Fig. 3 identify these areas as the LTRs of the B11 clone.

Comparison of the B11 and C60 maps shows complete concordance of sites on λ-B11 and λ-C60 inserts up to 1.0 kb from the left terminal *Eco*RI site. A region of divergence follows and spans 1.0–1.5 kb for λ-C60 and 1.5–2.0 kb for λ-B11; then there is a short stretch where three enzymes sites (*Pst* I, *Sst* I, and *Kpn* I) can be aligned at the first LTR of B11. A second region of nonhomology (≈1.5 kb for λ-C60 and 1.7 kb for λ-B11) follows. The rest of λ-C60 can be matched to two areas of λ-B11, at 5.3–7.0 kb units and 0.1 kb at the end of the B11 molecule. Therefore, the restriction enzyme maps are consistent with the heteroduplex result in defining a deletion (7.0–8.9 kb from the left end of the B11 clone and 3.4–5.3 kb from the 5′ end of linear SSAV) and two nonhomologous areas, one extending from and including the 5′ LTR and the adjacent 1.0 kb and another immediately before and excluding the 3′ LTR. Heteroduplex formation underestimates the latter substitution because this technique is less sensitive to small changes in base pair composition, which may alter restriction sites. As both of these regions on C60 represented potential SSV sarcoma-specific sequences, we proceeded to characterize them further.

λ-C60 Has an Inversion that Includes One LTR and Adjacent Sequences. Close examination of the C60 map suggested that the LTR at the 3′ end of the linear virus genome (left LTR of the permuted DNA) was maintained in the correct orientation and that the second LTR (identified by the *Pst* I, *Sst* I, and *Kpn* I sites) and 1.1 kb of adjacent sequences were inverted. The properly oriented 3′ LTR of C60 is identified by the *Pst* I, *Sst* I, and *Kpn* I sites at 2.5–2.9 kb of the C60 map. These same sites in reverse order are found at 4.1–4.4 kb of the C60 map. A 1.1-kb fragment homologous to the region to the right of the B11 5′ LTR is inverted in C60 and located between the direct and inverted LTRs on the map. This region is magnified and de-

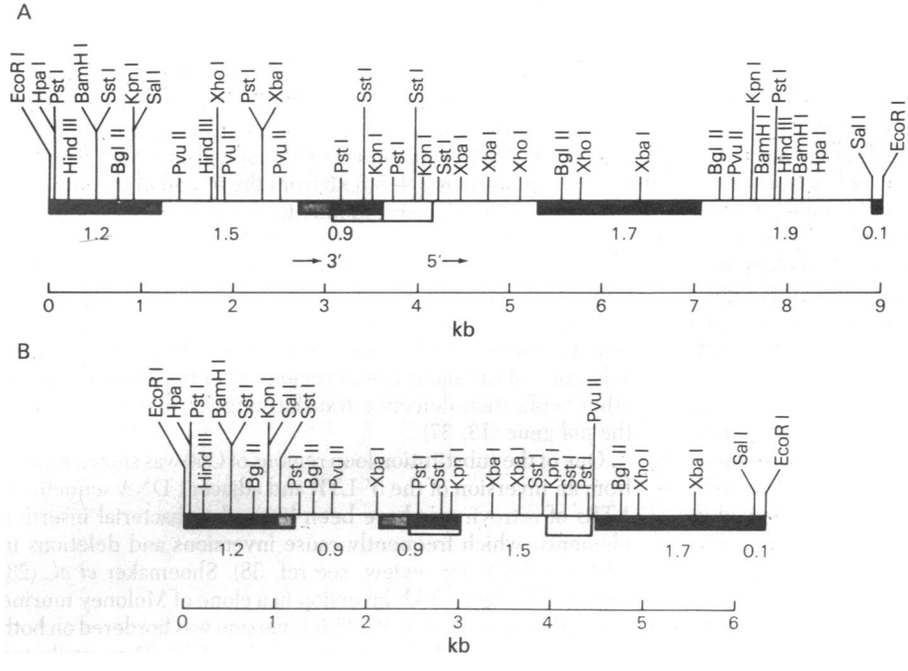
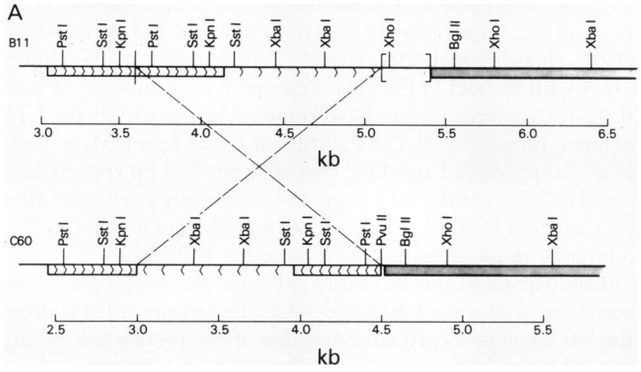


FIG. 3. Restriction enzyme maps of B11 (*A*) and C60 (*B*) clones. Dark bars indicate homology determined by restriction enzyme sites. Shaded extensions indicate homology implied by the heteroduplex in Fig. 1. Outlined boxes represent the LTR regions. Sections not highlighted represent the regions of substitution and deletion (right section of B11). Numbers are length measurements in kb that correspond approximately to the regions defined in the heteroduplex.



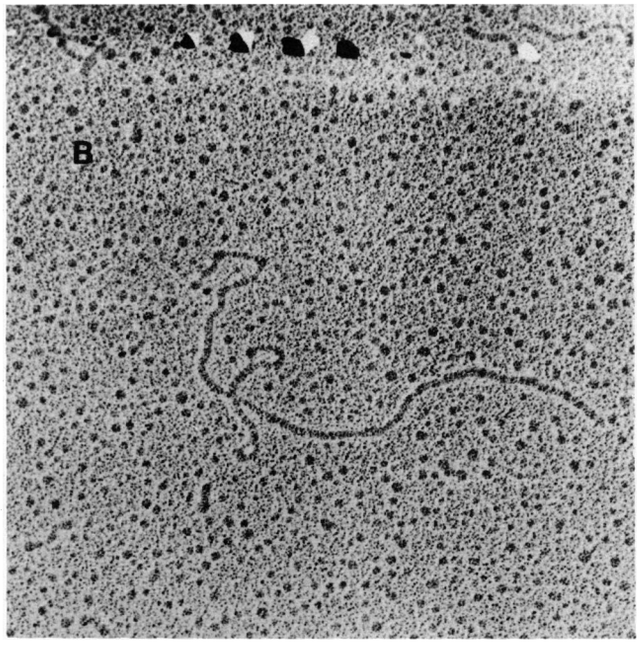


FIG. 4. (*A*) Restriction enzyme maps of the middle substitution loop region. Boxes with arrowheads indicate region and orientation of LTRs. Lines with arrows represent the portion of B11 inverted along with the right LTR in C60. The 0.3-kb bracketed region in B11 is deleted in C60. The shaded areas are homologous. (*B*) Duplex of rehybridized clone C60 excised from λ phage arms. Note persistence of both hairpins formed when the molecule was made single stranded. (×18,900.)

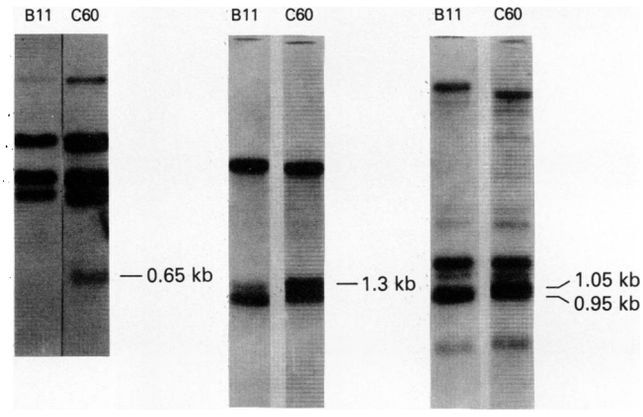


FIG. 5. Demonstration of C60-specific sequences. B11 and C60 insert DNA subcloned into pBR322 at the *Eco*RI site (pB11 and pC60, respectively) were ^{32}P-labeled by nick translation (36). λ-C60 recombinant phage DNA was digested with *Sal* I/*Pvu* II (*Left*), *Pst* I (*Middle*), or *Sal* I/*Xba* I (*Right*) and hybridized to either C60 or B11 probes by the Southern procedure (32). Fragments containing mostly C60-specific sequences are denoted.

picted in Fig. 4*A*. In addition to the inversion, a small region in B11 containing an *Xho* I site is missing in C60 and a new *Pvu* II site is found in C60 at the right-hand junction between the inversion and adjacent DNA. Although the *Pvu* II site arose as a consequence of the inversion, comparison with another cloned SSV DNA lacking an inversion shows the 300-bp region to be absent from it also. Thus, the 0.9-kb region of homology between the two substitution loops (Fig. 2*B*) includes at its right-hand end one LTR of C60. The middle substitution loop includes the second LTR of λ-B11 at the left end of one strand and the inverted LTR of λ-C60 at or near the right end of the other strand.

We can demonstrate the inversion of λ-C60 by electron microscopy. Self-hybridizing of single-stranded DNA that contains an inverted repeat should form a hairpin loop. We denatured C60 DNA purified from the phage vector arms and allowed it to rehybridize. This resulted in the molecule shown in Fig. 4*B*, which has hairpin loops in two annealed strands of C60. Because the isolation of cloned DNA from vector arms results in nicking of the insert we were unable to obtain accurate measurements of these hairpin structures and adjacent DNA. Heteroduplex formation between λ-C60 and Ch21A allows localization of the hairpin loop to the exact region of the LTRs in C60 (data not shown).

Demonstration of λ-C60-Specific Sequences: The 0.9-kb Substitution. B11 and C60 insert DNA were excised from λ DNA by digestion with *Eco*RI and subcloned into plasmid pBR322 at its sole *Eco*RI site. Each was then labeled with ^{32}P by nick translation and used as a probe for blot hybridization to restriction digests of λ-C60 (Fig. 5). We found that a 0.65-kb *Sal* I/*Pvu* II fragment, a 1.3-kb *Pst* I fragment, and a 1.05-kb *Sal* I/*Xba* I fragment were all detected readily with the homologous probe and barely detected with B11 [^{32}P]DNA. Both the *Sal* I/*Pvu* II and the *Sal* I/*Xba* I fragments contain common helper virus sequences in their left, 5′, ends, and the *Pst* I fragment contains ≈0.15 kb of the LTR at its 3′ end. Thus, part, if not all, of this 1.0- to 1.5-kb substitution is novel to C60.

DISCUSSION

We have derived molecular clones of SSAV and SSV. Restriction enzyme maps of the SSAV clone λ-B11 and unintegrated SSAV DNA from infected cells are identical except for permutation of λ-B11 at the *Eco*RI site. Two tandem units of direct LTR are identified in the middle of the permuted 9.0-kb cloned genome.

Comparison of the SSV clone λ-C60 with λ-B11 by heteroduplex formation and restriction enzyme mapping showed a large deletion and two areas of nonhomology in λ-C60. The 1.9-kb deletion maps at 3.4–5.3 kb from the 5′ end of the virus genome and probably corresponds to the *pol* gene. Deletion in this region is apparently not uncommon and not dependent on generation of sarcoma-specific sequences. Preliminary restriction analysis of one other clone from the defective "C" fragments obtained by preparative agarose gel shows that the clone represents defective SSAV DNA. It is identical to B11 except for a deletion of the same 1.9-kb region found in C60. Most of the other replication-defective transforming retroviruses also lack the *pol* gene (13, 37).

One of the substitution loop regions of C60 was shown to arise from an inversion of the 5′ LTR and adjacent DNA sequences. LTRs of retroviruses have been likened to bacterial insertion elements, which frequently cause inversions and deletions in adjacent DNA (for review, see ref. 38). Shoemaker *et al.* (23) reported finding a 3-kb inversion in a clone of Moloney murine leukemia virus (M-MuLV). This inversion was bordered on both sides by complete LTRs as is the case in λ-C60. They attributed

the inversion to self-integration of M-MuLV DNA, resulting in a duplication of 4 bp at the junction. In λ-C60, a novel *Pvu* II site is formed at the right-hand junction of the inversion.

Finally, we showed that the 0.9-kb substitution in C60, mapping in the 3'-terminal region, contains novel sequences. We believe this to be the SSV transformation-specific (*src*) gene. Although the final test for a *src* gene is its capacity for cellular transformation, the inversion in this molecule may greatly reduce its transforming efficiency. As *src*-specific sequences of the acutely transforming retroviruses are derived from cell DNA sequences (13–16), it was important to see whether the SSV-specific sequences also have normal cell counterparts. Indeed, we have detected specific hybridization of both ^{32}P-labeled C60 DNA and of a subclone containing part of the putative *src* region of C60 to DNA from many vertebrate species including man (unpublished data).

By extrapolation from the genetic maps of other helper leukemia viruses (39), we estimate the positions of *gag*, *pol*, and *env* genes to be at 0.8–3.2, 3.2–5.5, and 5.5–7.5 kb, respectively, on the linear SSAV genome. The 0.55-kb LTR sequences are located on both termini, and the extra 1.0-kb sequence to the right of the *env* gene may be similar to the *R* gene described for M-MuLV (40). SSV contains most of the *gag* gene intact (3.4 kb from the 5' end). The *pol* gene is largely deleted, and ≈1.3 kb of *env* gene is retained before the substitution. Thus, the gene order for SSV appears to be 5'-*gag*-Δ-*env*-*src*-C-3'. This structure is similar to that of Moloney murine sarcoma virus (41) and different from that of feline sarcoma virus (Snyder–Theilen) (39), Abelson leukemia virus (42), and the defective acutely transforming avian viruses (MC29, MH2, and CM-11) (43–45). The latter group of viruses have a structure of 5'-Δ-*gag*-*src*-Δ-*env*-C-3', and all synthesize a fused polyprotein containing part of the *gag* determinants and a polypeptide coded by the *src* or *onc* gene. We speculate that SSV would similarly code for a defective *gag* gene product and a *gag*-unrelated *src* protein. Autosera raised against SSV-transformed cells precipitated two polypeptides of 65,000 and 20,000 daltons and a high molecular weight glycoprotein from SSV-transformed nonproducer cells (J. Thiel and D. Bolognesi, personal communication). The availability of cloned SSV DNA and subclones derived from the putative *src* region should make it possible to determine which of these is the transforming protein of SSV.

Note Added in Proof. A clone of SSV isolated recently is identical to C60 but contains one LTR and has no inverted sequences. This cloned DNA transforms NIH/3T3 cells *in vitro*.

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1. Gallo, R. C. & Wong-Staal, F. (1980) in *Viral Oncology*, ed. Klein, G. (Raven, New York), pp. 399–431.
2. Deinhardt, F. (1980) in *Viral Oncology*, ed. Klein, G. (Raven, New York), pp. 357–398.
3. Theilen, G. H., Gould, D., Fowler, M. & Dungworth, D. L. (1971) *J. Natl. Cancer Inst.* **47**, 881–889.
4. Wolfe, L. G., Deinhardt, F., Theilen, G. H., Rabin, H., Kawakami, T. & Bustad, L. K. (1971) *J. Natl. Cancer Inst.* **47**, 1115–1120.
5. Wolfe, L. G., Smith, R. K. & Deinhardt, F. (1972) *J. Natl. Cancer Inst.* **48**, 1905–1908.
6. Todaro, G. J., Lieber, M. M., Benveniste, R. E., Sherr, C. J., Gibbs, C. J. & Gajdusek, D. C. (1975) *Virology* **67**, 335–357.
7. Wong-Staal, F., Gallo, R. C. & Gillespie, D. (1975) *Nature (London)* **256**, 670–672.
8. Reitz, M. S., Luczak, J. C. & Gallo, R. C. (1979) *Virology* **93**, 48–56.
9. Sherr, C. J., Lieber, M. M., Benveniste, R. E. & Todaro, G. J. (1974) *Virology* **58**, 492–503.
10. Benveniste, R. E. & Todaro, G. J. (1974) *Nature (London)* **252**, 456–459.
11. Lieber, M. M., Sherr, C. J., Todaro, G. J., Benveniste, R. E., Callahan, R. & Coon, H. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2315–2319.
12. Callahan, R., Mease, C. & Todaro, G. J. (1979) *J. Virol.* **30**, 124–131.
13. Bishop, J. M. (1978) *Annu. Rev. Biochem.* **47**, 34–88.
14. Stehelin, D., Varmus, H. I., Bishop, J. M. & Vogt, P. K. (1976) *Nature (London)* **260**, 170–173.
15. Frankel, A. E. & Fischinger, P. J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3705–3709.
16. Frankel, A. E., Gilbert, J. H., Porzig, C. J., Scolnick, E. M. & Aaronson, S. A. (1979) *J. Virol.* **30**, 821–827.
17. Hsu, T. W., Sabran, J. L., Mark, G. E., Guntaka, R. V. & Taylor, J. M. (1978) *J. Virol.* **28**, 810–818.
18. Hughes, S. H., Shank, P. R., Spector, D., Kung, H. J., Bishop, J. M., Varmus, H. E., Vogt, P. K. & Breitman, M. L. (1978) *Cell* **15**, 1397–1410.
19. Gilboa, E., Goff, S., Shields, A., Yoshimura, F., Mita, S. & Baltimore, D. (1979) *Cell* **16**, 863–874.
20. Benz, E. W. & Dina, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3294–3298.
21. Vande Woude, G. F., Oskarrson, M. K., Enquist, L. W., Nomura, S., Sullivan, M. & Fischinger, P. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4464–4468.
22. Dhar, R., McClements, N. L., Enquist, L. W. & Vande Woude, G. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3937–3941.
23. Shoemaker, C., Goff, S., Gilboa, E., Paskind, M., Mitra, S. W. & Baltimore, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3932–3936.
24. Tronick, S. R., Robbins, K. C., Canaani, E., Devare, S. G., Anderson, P. R. & Aaronson, S. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6314–6318.
25. McClements, W. L., Enquist, L. W., Oskarsson, M., Sullivan, M. & Vande Woude, G. F. (1980) *J. Virol.* **35**, 488–497.
26. Hirt, B. (1967) *J. Mol. Biol.* **26**, 365–367.
27. Wahl, G. M., Stein, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683–3687.
28. Vogelstein, B. & Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615–619.
29. Williams, B. G. & Blattner, F. R. (1979) *J. Virol.* **29**, 555–575.
30. Sternberg, N., Tiemeier, D. & Enquist, L. (1977) *Gene* **1**, 255–280.
31. Woo, S. L. C. (1980) *Methods Enzymol.* **68**, 389–395.
32. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
33. Taylor, J. M., Illmensee, R. & Summers, S. (1976) *Biochim. Biophys. Acta* **442**, 324–330.
34. Thomas, M. & Davis, R. W. (1975) *J. Mol. Biol.* **91**, 315–328.
35. Davis, R. W., Simon, M. & Davidson, N. (1971) *Methods Enzymol.* **24B**, 413–428.
36. Rigby, P. N., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 236–251.
37. Duesberg, P. H. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **44**, 13–29.
38. Calos, M. P. & Miller, J. H. (1980) *Cell* **20**, 579–595.
39. Sherr, C. J., Fedele, L. A., Oskarrson, M., Maizel, J. & Vande Woude, G. F. (1980) *J. Virol.* **34**, 200–212.
40. Sutcliffe, J. G., Shinnick, T. M., Verma, I. & Lerner, R. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3302–3306.
41. Donoghue, D. J., Sharp, P. A. & Weinberg, R. A. (1979) *J. Virol.* **32**, 1015–1027.
42. Shields, A., Goff, S., Paskind, M., Otto, G. & Baltimore, D. (1979) *Cell* **18**, 955–962.
43. Bister, K., Hayman, M. J. & Vogt, P. K. (1977) *Virology* **82**, 431–448.
44. Bister, K., Loliger, H. & Duesberg, P. H. (1979) *J. Virol.* **32**, 208–219.
45. Mellon, P., Pawson, A., Bister, K., Martin, G. S. & Duesberg, P. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5874–5878.