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Isolation of an SSAV-Related Endogenous Sequence from Human DNA

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We have found human DNA to contain a number of sequences related to simian sarcoma associated virus (SSAV). One of these sequences was isolated from a human genomic library. The molecular clone, termed S71, contains regions homologous to SSAV *gag* and *pol* fragments and SSAV LTR. Furthermore, hybridization experiments and DNA sequencing revealed distinct homologies to the reverse transcriptase coding region of several other retroviruses including baboon endogenous virus (BaEV) and murine leukemia viruses (MuLV) as well as retrovirus-like elements. Some sequence homology was also found with the C-type retrovirus-related multicopy human clone 4-1. S71 is present in only one copy per human genome equivalent and exhibits an *Eco*RI restriction fragment length polymorphism.

INTRODUCTION

Endogenous retroviral sequences are present in the DNA of many vertebrate species including birds, rodents, and primates (Stoye and Coffin, 1985). Some of them represent full-length retroviral genomes, others are incomplete and lack LTRs or *env* sequences or other parts of the viral genome. Although most endogenous retroviruses are defective, some have been shown to be pathogenic for their hosts. They represent a reservoir of viral genes which can be activated spontaneously, by recombination events, or by physical or chemical agents. In some mouse strains, e.g., activated endogenous retroviruses induce hematopoietic proliferative diseases.

In human DNA retrovirus-like elements have also been detected by low stringency hybridization technics. Two families of Moloney MuLV-related sequences have been isolated from a human library by employing an African green monkey endogenous retroviral probe (Martin *et al.*, 1981). Both families are highly related and occur in numerous copies in the human genome. One family contains only *gag* and *pol* sequences, whereas the other class of clones appears to contain complete retroviral genomes (Repaske *et al.*, 1983; Steele *et al.*, 1984). However, nucleotide sequencing revealed that these proviruses are also defective (Repaske *et al.*, 1985) and RNA transcripts found in human placenta, colon carcinoma, and breast carcinoma cells contain in-frame termination codons (Rabson *et al.*, 1983, 1985).

Other reports have described the isolation of two human BaEV-related clones, ERV1 and ERV3, using an endogenous chimpanzee provirus for screening (Bonner *et al.*, 1982; O'Brien *et al.*, 1983; O'Connell *et al.*, 1984). The ERV1 and ERV3 proviruses are only present in one copy per genome equivalent and were mapped to chromosome 18 and 7, respectively. Several different human BaEV-related clones have been isolated by nonstringent hybridization with BaEV-LTR (Noda *et al.*, 1982). In addition to these human sequences related to C-type viruses, isolation of clones partly homologous to A-, B-, and D-type retrovi-

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ruses has also been reported (Callahan *et al.*, 1982, 1985; Westley and May, 1984).

Studies using cross-reactive antibodies indicate that human endogenous retroviral sequences may be expressed on the protein level. Antibodies against a synthetic peptide derived from *gag*-related sequences of the human retroviral clone ERV1 detect a 75,000-Da protein in normal human placenta and in some human tumors (Suni *et al.*, 1984; Wahlström *et al.*, 1985). Expression of C-type retrovirus related proteins has also been studied by several investigators using antibodies against retroviral structural proteins. An SSAV/GaLV (gibbon ape leukemia virus) p30-cross-reacting protein with a molecular weight of 30,000 has been found in human leukemic cells (Derks *et al.*, 1982) and placenta (Jerabek *et al.*, 1984). We have reported the isolation of a 70,000-Da protein from human leukemic sera that cross-reacts with p30 from the SSAV/GaLV primate retrovirus group and p30 from BaEV (Hehlmann *et al.*, 1983), and the detection of SSAV gp70-related antigens in leukemic and nonleukemic sera (Hehlmann *et al.*, 1984; Schetters *et al.*, 1985). Here we report the detection and isolation of SSAV-homologous endogenous sequences from human DNA which may possibly encode SSAV-related polypeptides.

MATERIALS AND METHODS

Materials

The plasmid pB11 containing the full-length SSAV genome and molecular clones of human T-cell lymphotropic virus (HTLV) strain I, II, and III were obtained from R. C. Gallo, NCI, Bethesda. Further molecular clones used for hybridization experiments contained DNA from BaEV M7 (Battula *et al.*, 1982); the AKR ecotropic provirus (AKV) (Lenz *et al.*, 1982); mouse virus-like 30 S elements (VL30) (Courtney *et al.*, 1982); ERV1 (Bonner *et al.*, 1982); and clone 4-1 (Steele *et al.*, 1984). Moloney murine leukemia virus (Mo-MuLV) DNA was provided by P. G. Strauss, GSF, Neuherberg. The recombinant DNA library Ly 66 from a human Burkitt lymphoma celline was a gift from M. Lipp, University of Munich.

Nucleic Acid Hybridization

DNA cleaved by restriction enzyme digestion was separated in 0.6–1.6% agarose gels and transferred to nitrocellulose membranes by the procedure of Southern (1975). DNA for hybridization probes was excised from the plasmid, separated from the vector by preparative gel electrophoresis, and purified by ion exchange chromatography (NACS-52 from BRL, Maryland). ^{32}P-labeled probes were made by nick translation (Rigby *et al.*, 1977), or for small DNAs by end-filling using the Klenow fragment of *Escherichia coli* DNA polymerase I (Telford *et al.*, 1979). Base mismatch for high stringency and low stringency hybridization conditions was calculated according to Howley *et al.* (1979).

High stringency hybridization (84% homology for hybridization, 94% homology for high temperature wash step). Nitrocellulose filters were prehybridized for at least 2 hr at 42° in 50% formamide, 5× SSC (1× SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate), 5× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll), 0.05 *M* sodium phosphate, 1% glycine, and 0.2 mg/ml denatured salmon sperm or *E. coli* DNA. Hybridization was carried out for at least 20 hr at 42° in 50% formamide, 5× SSC, 5× Denhardt's solution, 0.02 *M* sodium phosphate, 5% dextransulfate (optional), 50 μg/ml denatured salmon sperm DNA, and 1–2 × 10^6 cpm/ml of denatured ^{32}P-labeled DNA (sp act 3–5 × 10^8 cpm/μg). Filters were washed at room temperature for 30 min in 2× SSC, 0.5× Denhardt's solution, for 15 min in 2× SSC, 0.1% SDS, and at 65° for 2 hr in 0.1× SSC, 0.1% SDS.

Low stringency hybridization (72% homology for hybridization and wash). Filters were prehybridized and hybridized as described for high stringency conditions, with the exception that the hybridization solutions contained 40% formamide and 6× SSC, and that the temperature was lowered to 35°. Filters were washed under low stringency conditions at room temperature

for 30 min in 2× SSC, 0.5× Denhardt's solution, for 15 min in 2× SSC, 0.1% SDS, and at 55° for 2 hr in 2× SSC 0.1% SDS. In some cases, filters were rewashed under middle stringency conditions at 55° for 30–60 min in 0.5× SSC, 0.1% SDS, and highly stringent at 65° for 1 hr in 0.1× SSC, 0.1% SDS. Bands were visualized by autoradiography at −70° for 1–14 days using Kodak XAR-5 film and an intensifying screen.

Screening of a Human Recombinant DNA Library

A once-amplified library from a human Burkitt lymphoma celline cloned in λ vector Embl 3A was used for screening. Phages (1×10^6) were plated on *E. coli* LE 392. Phages were screened by the procedure of Benton and Davis (1977). Duplicate nitrocellulose filters were hybridized with a 9.0-kb fragment containing the full-length SSAV genome and a 2.2-kb *pol-env* fragment using low-stringency conditions as described above. The hybridization solution contained 50 μg/ml denatured *E. coli* DNA and 0.3 μg/ml λ DNA for reduction of background. After exposure filters were rewashed for 1 hr at 55° in 0.5× SSC, 0.1% SDS to raise stringency and exposed again. For rescreening of the newly plated library with the isolated clone S71 hybridization was carried out under high stringency conditions as described for Southern blots.

DNA Sequence Analysis

Subfragments of clone S71 used for hybridization experiments and DNA sequencing were cloned in pUC9 (Vieira and Messing, 1982). Dideoxy sequencing of double stranded plasmid DNA was carried out as described by Chen and Seeburg (1985) with some modifications (P. Heinrich, University of Munich, personal communication).

RESULTS

Identification of SSAV-Related Sequences in Human Cellular DNA

Human cellular DNA prepared from two individuals was digested with *Eco*RI, *Hin*dIII, and *Bam*HI (Fig. 1). The DNA of

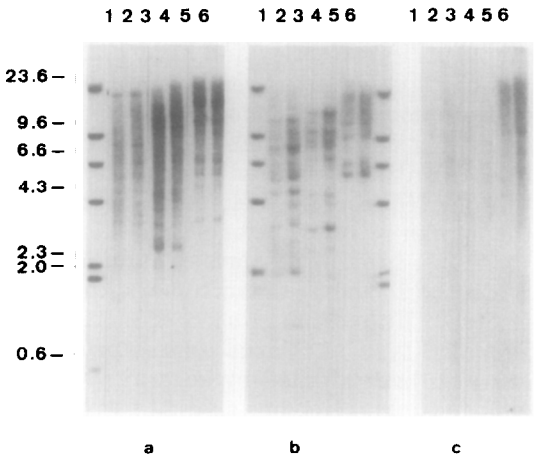

FIG. 1. Detection of SSAV-related sequences in human genomic DNA. Human DNA from two individuals (lanes 1, 3, and 5, a patient with chronic myelogenous leukemia; lanes 2, 4, and 6, a nonleukemic person) was digested with *Eco*RI (lanes 1, 2), *Hin*dIII (lanes 3, 4), and *Bam*HI (lanes 5, 6), separated in a 0.6% agarose gel, and transferred to nitrocellulose filters. SSAV-related sequences were detected by hybridization with ^{32}P-labeled total SSAV DNA (a), a 1.2-kb SSAV *pol* fragment (b), and a 0.4-kb SSAV *env* fragment (c) under low stringency conditions as described under Methods.

lanes 1, 3, and 5 was isolated from the spleen of a patient with chronic myelogenous leukemia, whose serum contained a high amount of SSAV-related proteins (Hehlmann *et al.*, 1983, 1984). The DNA of lanes 2, 4, and 6 was prepared from white blood cells of a nonleukemic person. The DNA was hybridized under low stringency conditions (72% homology) with a 9.0-kb fragment containing the whole SSAV genome, and with subgenomic fragments of SSAV containing *pol* and *env* sequences (Fig. 2). The pattern obtained with each hybridization probe consists of multiple discrete bands in addition to a smear. Hybridization with the 1.2-kb *pol* fragment of SSAV (Fig. 1b) yielded the clearest pattern of prominent bands. Most of the *pol*-related fragments remained visible when the filters were rewashed at middle stringency (0.5× SSC, 0.1% SDS, 55°; data not shown). This is not surprising because the *pol* region of retroviruses contains highly conserved DNA sequences (Chiu *et al.*, 1984).

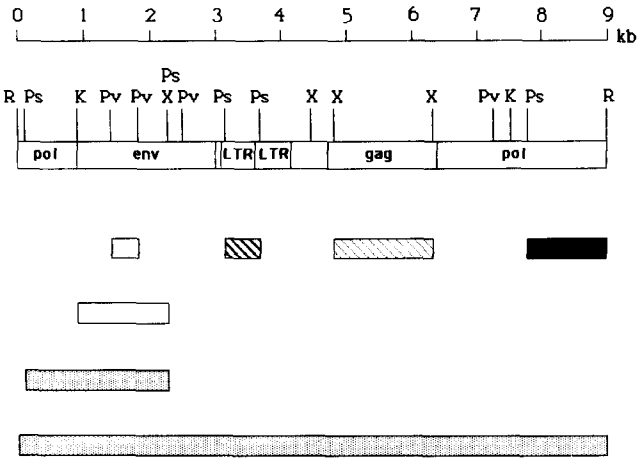

FIG. 2. SSAV fragments used for hybridization. The restriction enzyme map shows the full-length SSAV genome of the molecular clone pB11 (Gelmann *et al.*, 1981). Abbreviations: K, *Kpn*I; Ps, *Pst*I; Pv, *Pvu*II; R, *Eco*RI; *X*, *Xba*I.

The human DNA hybridizes also weakly with a 0.4-kb *env* fragment (Fig. 1c), although the *env* gene is known to comprise the most specific region of the retroviral genome. A middle stringent wash, however, eluted most of the hybridized *env* DNA (data not shown). In all three hybridization patterns no differences are visible between both human DNA samples, indicating that human DNA contains endogenous SSAV related sequences.

Isolation of a Molecular Clone Containing SSAV-Related Human Sequences

A human recombinant DNA library was screened using the full-length SSAV genome and several subgenomic fragments as hybridization probes (Fig. 2). One molecular clone (S71) was isolated that reacted specifically with total SSAV DNA and a 2.2-kb *pol-env* subfragment under low stringency and middle stringency hybridization conditions. SSAV subfragments smaller than 0.5 kb, although hybridizing with human DNA in Southern blots, did not lead to reproducible screening results. Figure 3 shows a partial restriction enzyme map of S71. The positions of the restriction enzyme cleavage sites were determined by Southern blot analysis of single and double digests of S71 by hybridizing with S71 fragments as probes (data not shown). The library was rescreened with the 9.6-kb *Sal*I fragment of S71 (Fig. 3, position 13.6-23.2 kb) at high stringency. Beside four clones identical with S71 we obtained five new clones (S71-1-S71-5, Fig. 3) with overlapping restriction enzyme patterns. Together with S71 the new clones represent about 36 kb of a single genomic locus. Since no variation in the S71 restriction enzyme cleavage pattern could be detected in 15 clones isolated from 10^6 recombinant phages, we assume that S71 occurs in only one copy per genome equivalent. The restriction enzyme pattern differs completely from that of BaEV and Mo-MuLV C-type virus-related human endogenous sequences (Noda *et al.*, 1982; Bonner *et al.*, 1982; O'Connell *et al.*, 1984; Steele *et al.*, 1984).

Identification of Retroviral Genes

DNA from clone S71 was digested with various restriction enzymes and subsequently hybridized with total SSAV DNA as well as subgenomic fragments (Fig. 2) under low stringency conditions. Figure 4 shows comparative exposures of the hybridization patterns obtained with total SSAV DNA (a), a 1.2-kb fragment of the *pol* gene (b, c, and d), a 1.6-kb fragment of the *gag* gene (f), and a 0.6-kb fragment

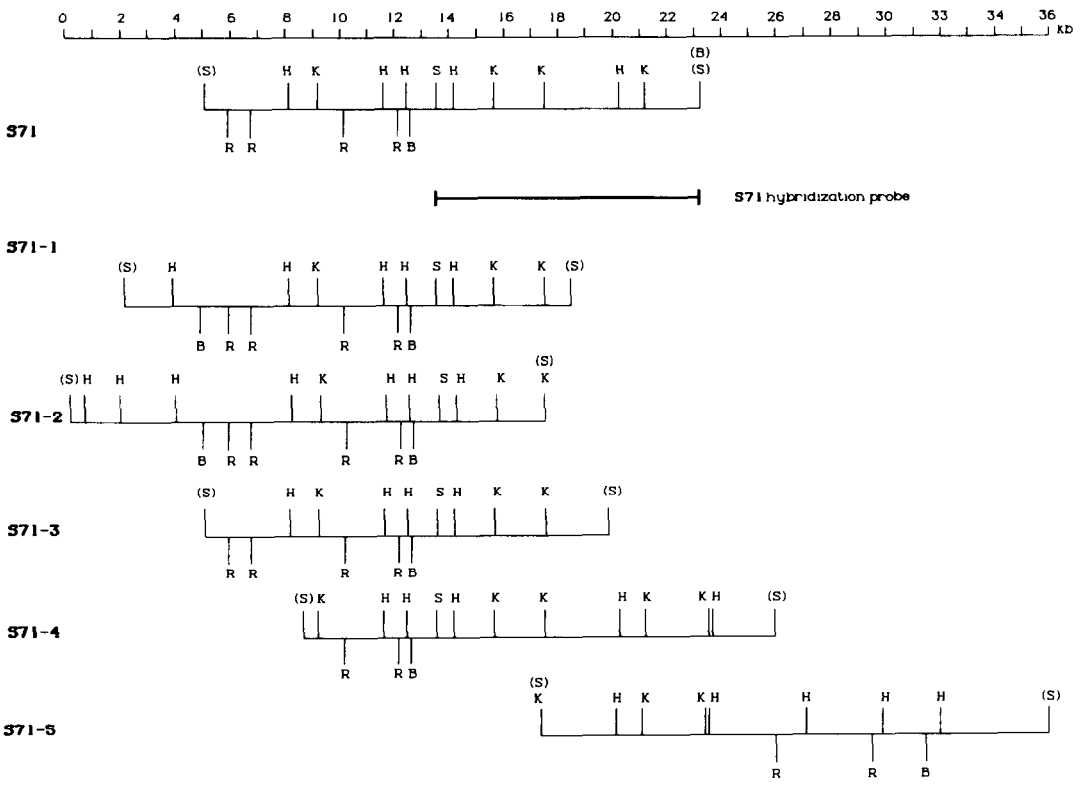

FIG. 3. Restriction enzyme maps of S71 molecular clones. Abbreviations: B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I; R, *Eco*RI; S, *Sal*I. The restriction enzyme sites in parentheses are generated by vector sequences.

containing the LTR (e). The region homologous to SSAV comprises about 6 kb of S71 (Fig. 5). Hybridization with the 1.2 kb *pol* probe yielded the greatest intensity which remained almost unchanged after washing at middle stringency conditions (Figs. 4b, c). Of all SSAV probes used, only the band obtained with the 1.2-kb *pol* fragment was still visible after a high stringency wash (Fig. 4d). Somewhat weaker hybridization was found with the SSAV LTR probe (Fig. 4e). The weakest hybridization was obtained with the 1.6-kb *gag* fragment (Fig. 4f). In the latter case the respective S71 bands disappeared after middle stringency washing. Probes from the envelope region of SSAV (Fig. 2) did not hybridize visibly with S71 (data not shown). The relative positions of the S71 restriction enzyme fragments hybridizing with SSAV *gag*, *pol*, and LTR sequences are shown in Fig. 5. The SSAV-related sequences of S71 are flanked by sequences hybridizing strongly with the human Alu probe BLUR8 (Jelinek *et al.*, 1980). Reciprocal hybridization of several digests of the molecularly cloned SSAV genome (pB11) was carried out with the radiolabeled 9.6-kb *Sal*I fragment of S71. The result also reveals a strong homology of S71 to the *pol* sequences of SSAV (Fig. 6a) and confirms the above data. Hybridization of other retroviral clones with a 3-kb *Hin*dIII–*Bam*HI fragment (position 20.2–23.2, Fig. 5), comprising the *pol*-LTR-related sequences of S71, showed, compared to SSAV, a somewhat weaker homology to the *pol* region of the endogenous primate retrovirus BaEV, the ecotropic murine AKV virus, and Moloney MuLV, and a very weak homology to mouse VL30 elements (Fig. 6b). No hybridization was observed with the HTLV-I, II, and III genomes and

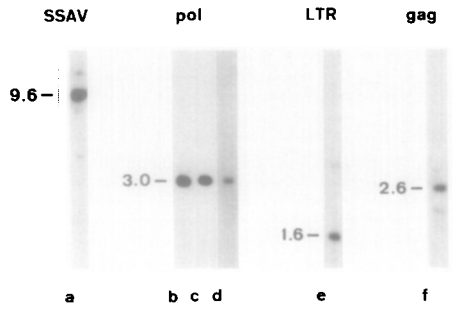

FIG. 4. Detection of SSAV-related sequences in S71 DNA. The molecular clone S71 was digested with *Sal*I (a), *Bam*HI/*Hin*dIII (b–d), *Pst*I/*Sal*I (e), and *Hin*dIII/*Kpn*I (f), electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose filters. SSAV-related sequences were detected by hybridization with ^{32}P-labeled total SSAV DNA (a), a 1.2-kb SSAV *pol* fragment (b–d), a 0.6-kb SSAV LTR fragment (e), and a 1.6-kb SSAV *gag* fragment (f) under low stringency conditions. Nitrocellulose filters were washed at low stringency (a, b, e, f), and rewashed at middle stringency (c), and high stringency (d) as described under Methods.

Rous sarcoma virus DNA (data not shown). An ERV1 subclone (HC55p4) comprising the 3′ end of *pol* and *env*, LTR, and flanking cellular sequences and a 4-1 subclone (pH6env) containing 3′ *pol* and 5′ *env* of Mo-MuLV related human sequences (Steele *et al.*, 1984) failed to hybridize with S71 (data not shown). However, a partial homology of S71 to ERV1 and clone 4-1 cannot be ruled out by this experiment, since the subclones lacked the whole *gag* gene and a large part of *pol* sequences which would be expected to exhibit the greatest homology.

Nucleotide Sequences of S71 pol Region

For further characterization portions of the S71 *pol*-related region were subjected to sequence analysis (Fig. 7). Since DNA sequences of SSAV or GaLV are not available for comparison, we aligned the S71 sequences with the published sequences of the human clone 4-1 (Repaske *et al.*, 1985) and the murine leukemia virus AKV (Etzerodt *et al.*, 1984). S71 shows significant nucleotide homology to the *pol* gene of clone 4-1 and AKV over the whole range analyzed. The corresponding sequences comprise the 3′ half of the coding region for reverse transcriptase between nucleotide 3786 and 4574 in AKV. The S71 sequences shown in Fig. 7 seem to be part of an open reading frame. Comparison of the deduced amino acid sequence of S71 revealed a highly conserved region with 52% homology to clone 4-1 and AKV between nucleotides 3786 and 3983 (Fig. 7a). The amino acid sequence of S71 shown in Fig. 7b is 54% homologous to AKV but only 26% homologous to clone 4-1. S71 region c (Fig. 7c) was found to be 47% homologous to AKV and 39% homologous to clone 4-1. Significantly less homology was found with HTLV-III/LAV (Fig. 7b). The S71 sequences are separated by approximately the same spaces as the homologous sequences in AKV, indicating colinearity between S71 and AKV *pol* gene in that region.

Hybridization of Human DNA with S71

Human genomic DNA was cleaved with various restriction enzymes and hybridized to the 9.6-kb *Sal*I fragment of S71 (Fig. 5,

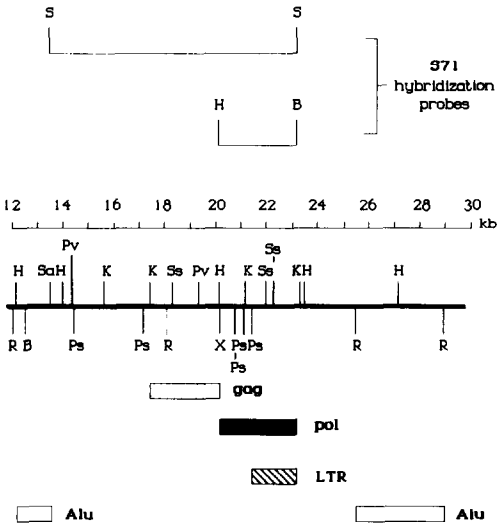

FIG. 5. Identification of SSAV-related sequences in S71 DNA. Regions of homology between S71 and SSAV fragments are represented by bars carrying the same markings as the respective SSAV fragments used as hybridization probes (Fig. 2). Abbreviations: H, *Hin*dIII; K, *Kpn*I; Ps, *Pst*I; Pv, *Pvu*II; R, *Eco*RI; Ss, *Sac*I; X, *Xba*I.

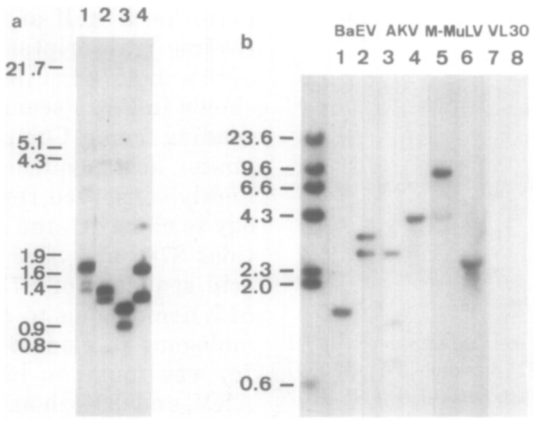

FIG. 6. (a) Reciprocal hybridization of SSAV with S71 DNA. The molecular clone pB11 (Fig. 2) was cleaved with *Bgl*II/*Sac*I (1), *Kpn*I/*Eco*RI (2), *Hin*dIII/*Eco*RI (3), and *Pvu*II/*Eco*RI (4) and analyzed by Southern blotting as described. The filter was hybridized under low stringency conditions with ^{32}P-labeled S71 9.6-kb *Sal*I fragment as probe. (b) Hybridization of cloned retroviruses with S71 DNA. BaEV DNA was digested with *Bam*HI/*Eco*RI (1) and *Hpa*I/*Xho*I (2), AKV DNA with *Hpa*I/*Pst*I (3) and *Kpn*I/*Pst*I (4), Mo-MuLV DNA with *Sal*I (5) and *Bam*HI/*Pst*I (6), and VL30 DNA with *Xho*I (7) and *Hin*dIII (8), and analyzed by Southern blotting. Hybridization was carried out under low stringency conditions with ^{32}P-labeled S71 3-kb *Hin*dIII-*Bam*HI fragment.

position 13.6-23.2) under high stringency conditions. Digestion with *Bam*HI resulted in one band corresponding to a molecular weight of 18 kb, digestion with *Hin*dIII in two bands of 6.4 and 3.3 kb, digestion with *Kpn*I in four bands of 6.7, 3.7, 2.2, and 1.9 kb, and double digestion with *Kpn*I and *Hin*dIII yielded four bands of 2.8, 2.2, 1.9, and 1.7 kb (Fig. 8a). These lengths are in agreement with the molecular weights determined for the restriction enzyme fragments of the isolated S71 clones (Fig. 3). These results indicate that S71 occurs in only one copy per human genomic DNA equivalent as suggested previously by our screening data. However, digestion with *Eco*RI yielded beside the expected 13.5-kb fragment two additional fragments 6.3 and 6.8 kb in length (Fig. 8b, lane 6). This finding might be explained by the presence of one or two additional copies of sequences closely related to S71. The other possibility would be a restriction fragment length polymorphism created by an additional *Eco*RI site within the 13.5-kb fragment in one parental chromosome. To test this latter hypothesis we hybridized *Eco*RI/*Hin*dIII double digests of human genomic DNA with the same 9.6-kb S71 probe. The 6.4- and 3.3-kb bands obtained correspond to the fragments found in the *Hin*dIII digest (Fig. 8a, lane 2; Fig. 5, position 14.1-20.2 and position 20.2-23.6 kb). The additional 4.0- and 2.4-kb fragments add up to the length of the smaller *Eco*RI fragment (6.3 kb). To determine the position of the additional *Eco*RI site, *Eco*RI and *Eco*RI/*Hin*dIII digests of human genomic DNA were hybridized with the 3-kb *Hin*dIII-*Bam*HI fragment of S71 (Fig. 5). In the track containing the *Eco*RI digest only the 13.5- and the 6.8-kb fragments were visible (Fig. 8c, lane 7), whereas the 6.3-kb fragment could not be detected. The *Eco*RI/*Hin*dIII double digest showed only the 3.3-kb *Hin*dIII fragment (lane 8). This confirms our assumption of a restriction enzyme polymorphism rather than the possibility of several different gene loci. Therefore we placed the internal *Eco*RI site at position 18.2 (Fig. 5). We conclude from these results that S71 is present at one single gene locus in the human genome. In both human DNA preparations analyzed by Southern blotting S71 exhibits an *Eco*RI restriction fragment length polymorphism that could not be detected in the human library used for screening (Fig. 3).

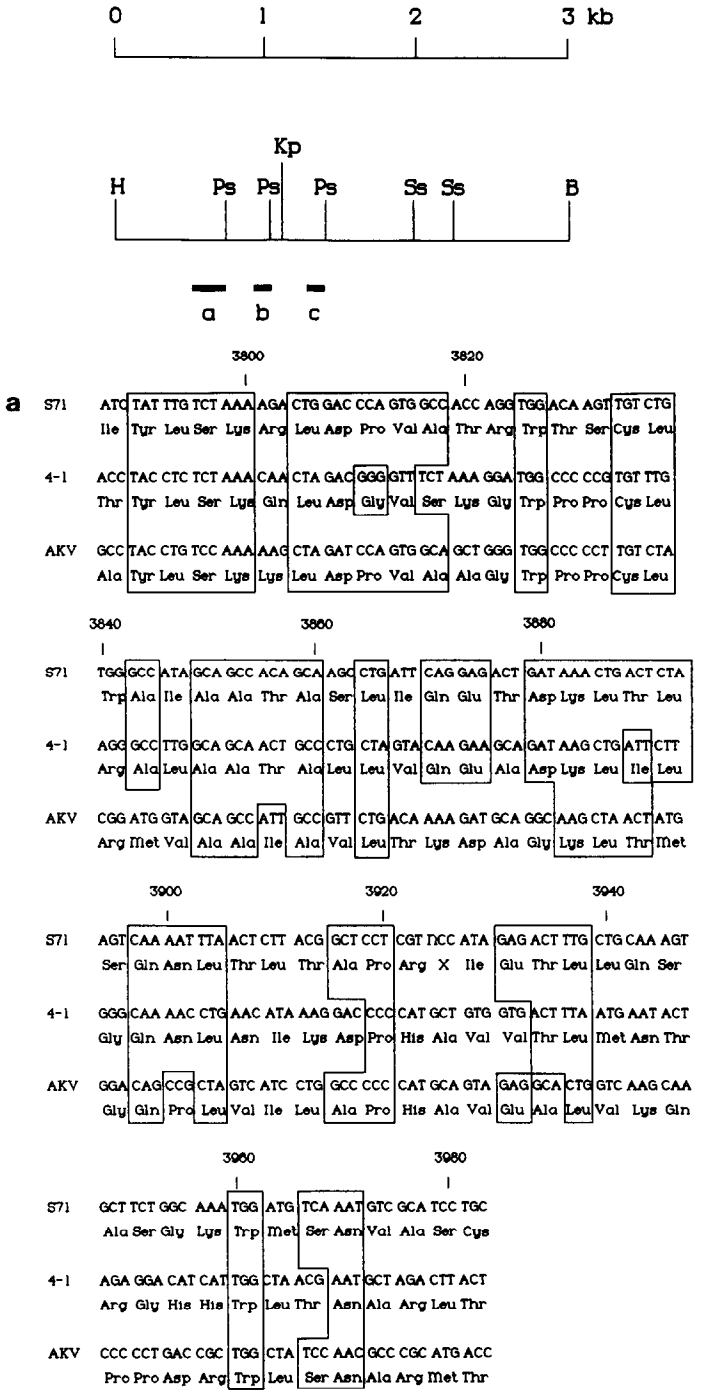

FIG. 7. Nucleotide sequences of S71 *pol* region. S71 nucleotide sequences of the segments indicated and deduced amino acid sequences were compared with those of clone 4-1 (Repaske *et al.*, 1985), AKV (Etzerodt *et al.*, 1984), and HTLVIII/LAV (HIV) (Ratner *et al.*, 1985) by computer-assisted alignement (PIR Alignement Score Program). Boxes indicate amino acid homologies with S71. Numbering of the bases corresponds to the sequence of AKV (Etzerodt *et al.*, 1984).

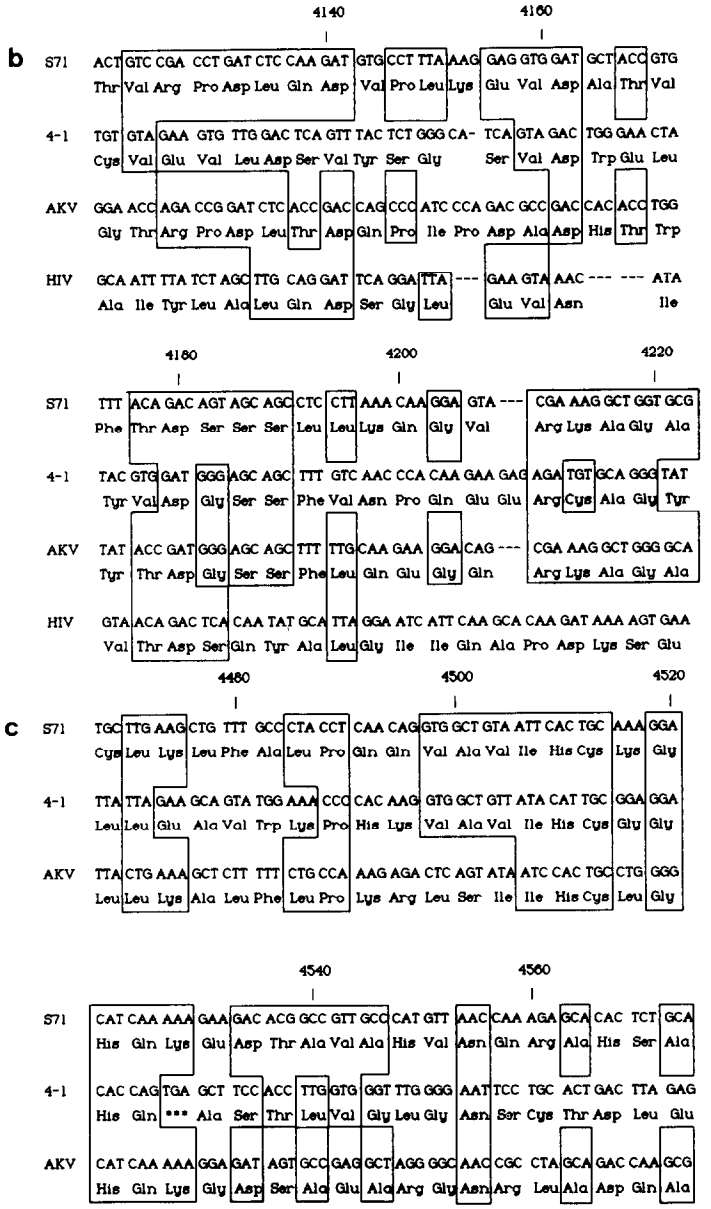

FIG. 7—*Continued.*

DISCUSSION

This study demonstrates the presence of SSAV-related sequences in human DNA. We have identified sequences hybridizing to full-length SSAV DNA and to subgenomic *pol* and *env* fragments of SSAV under low stringency conditions. Furthermore a recombinant clone (S71) was isolated from a human DNA library using the same hybridization conditions. This clone contains sequences related to *gag*, *pol*, and LTR sequences of SSAV. The *gag*- and LTR-related sequences exhibit a homology to SSAV DNA of up to 70–80% estimated by the method of Howley *et al.* (1979). The *pol*-related sequences of S71 hybridize with a homology of probably more than 80% to

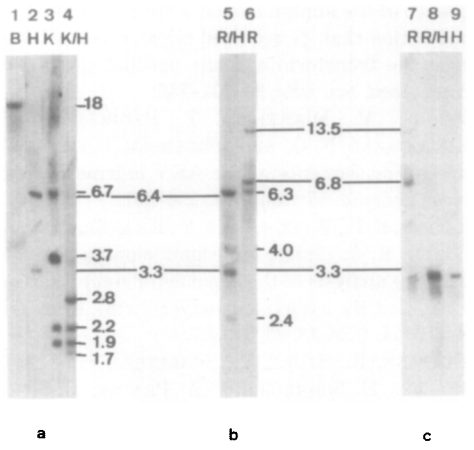

FIG. 8. Identification of S71 homologous sequences in human DNA. Human genomic DNA was digested with *Bam*HI (1), *Hin*dIII (2, 9), *Kpn*I (3), and *Eco*RI (6, 7) and double digested with *Hin*dIII/*Kpn*I (4), and *Hin*dIII/*Eco*RI (5, 8), separated in 0.6% agarose gels, and transferred to nitrocellulose filters. Filters were hybridized with ^{32}P-labeled S71 9.6-kb *Sal*I fragment (a, b) and S71 3-kb *Hin*dIII–*Bam*HI fragment (c) under high stringency conditions as described under Methods.

an SSAV *pol* fragment from the coding region of the viral reverse transcriptase (Gelmann *et al.*, 1981; Devare *et al.*, 1983). Nucleotide sequence analysis of portions of the S71 *pol*-related sequences indicates an overall polynucleotide and deduced amino acid homology in the range of 40–55% with the 3′ half of the murine leukemia virus AKV reverse transcriptase coding region (Etzerodt *et al.*, 1984) and 25–50% with the corresponding region of the human clone 4-1 (Repaske *et al.*, 1985). The restriction enzyme cleavage pattern of S71 differs completely from that of the BaEV- and Mo-MuLV-related endogenous human proviral sequences (Noda *et al.*, 1982; Bonner *et al.*, 1982; O'Connell *et al.*, 1984; Steele *et al.*, 1984). Moreover DNA probes from ERV1 (Bonner *et al.*, 1981) and clone 4-1 (Steele *et al.*, 1984) containing 3′ *pol*, *env*, and LTR sequences do not hybridize with S71. Therefore we conclude that S71 may represent a new class of human endogenous C-type related retroviral elements. S71 cannot contain a full-length retroviral genome because the retrovirus-related sequences comprise a maximum of 6 kb. Furthermore only one region homologous to SSAV LTR was found and *env*-related sequences could not be detected. Rescreening of the human library with S71 DNA and Southern blot hybridization of different human DNA preparations suggest that S71 represents one single human gene locus. With the exception of one *Eco*RI site the restriction enzyme pattern of S71 is identical in all three human DNAs examined. These data indicate that S71 may be an ancient integration site of a retrovirus that lost parts of its genome during evolution. A further possibility is that endogenous retroviral elements like S71 may represent progenitors from which retroviral genomes may have evolved (Temin, 1980). A possible role of cellular retrovirus-like elements has also been demonstrated in the last years by the detection of processed pseudogenes and highly repeated short DNA segments, as for instance human Alu sequences, which must have been generated by reverse transcription (Baltimore, 1985). Reverse transcriptase coding sequences have been detected in movable cellular elements like copia and yeast transposons and in the introns of yeast mitochondrial genes (Michel and Lang, 1985). Moreover reverse transcriptase activity has been demonstrated for the Ty element of yeast (Boeke *et al.*, 1985). Retrovirus-like elements containing reverse transcriptase genes may therefore represent intermediates between cellular transposons and retroviruses.

The possible role of SSAV-related endogenous sequences in human leukemia, indicated by our previous findings of SSAV-related antigens in human leukemic sera, remains still unclear. We could not detect any differences between the hybridization patterns of DNA from a leukemic and a nonleukemic person using SSAV or S71 DNA as probe. Therefore we assume that a possible contribution to the course of the disease may happen on the level of expression. The presence of LTR related sequences indicate that S71 may have the potential to activate cellular oncogenes by translocation or to express retrovirus-related proteins. The possible association between the human SSAV-related proteins

and the SSAV-related human sequences represented by S71, as well as their possible involvement in human neoplasias, remains an issue of further investigation.

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