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Nucleotide sequence of the simian sarcoma virus genome: Demonstration that its acquired cellular sequences encode the transforming gene product p28^{sis}

(primate retrovirus/antisera to synthetic peptides/transforming protein)

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ABSTRACT **The complete nucleotide sequence of the proviral genome of simian sarcoma virus (SSV), an acute transforming retrovirus of primate origin, has been determined. Like other transforming viruses, SSV contains sequences derived from its helper virus, simian sarcoma-associated virus (SSAV), and a cell-derived (v-*sis*) insertion sequence. By comparison with the sequence of Moloney murine leukemia virus, it was possible to precisely localize and define sequences contributed by SSAV during the generation of SSV. Comparative sequence analysis of SSV and SSAV showed that SSAV provides regulatory sequences for initiation and termination of transcription of the SSV transforming gene. Moreover, coding sequences for the putative protein product of this gene appear to initiate from the amino terminus of the SSAV *env* gene. Antibodies to synthetic peptides derived from the carboxy and amino termini of the putative protein predicted by the open reading frame identified within v-*sis* specifically detect a M_r 28,000 protein, p28^{sis}, in SSV-transformed cells. These and other findings confirm the predicted amino acid sequence of this protein and localize it to the coding region of the SSV transforming gene.**

Simian sarcoma virus (SSV) is the only primate representative of the class of replication-defective acute transforming retroviruses (1). This virus arose by recombination of the nondefective simian sarcoma-associated virus (SSAV) with cellular sequences (*sis*) present within the woolly monkey genome (2–5). By deletion mutant analysis, v-*sis* sequences have been shown to be essential for SSV transforming activity (6). In an effort to better understand its structural organization as well as the molecular mechanisms involved in SSV transformation, we have determined the primary sequence of the complete SSV genome and its rat cellular flanking sequences. The results provide insight into the mechanisms of the transcription and translation of the SSV transforming gene. Moreover, these studies show that a M_r 28,000 product (p28^{sis}) detected in SSV-transformed cells with antibodies to synthetic peptides originates from v-*sis* coding sequences in the SSV transforming region.

MATERIALS AND METHODS

Molecular Cloning of SSV DNA. The isolation of a molecular clone of integrated SSV DNA from SSV-11 nonproductively transformed normal rat kidney cells in Charon 16A phage (2) and its subsequent subcloning in pBR322 (7) has been described. The 5.8-kilobase pair (kbp) SSV DNA insert from plasmid DNA was purified by agarose gel electrophoresis and DEAE-cellulose (DE-52, Whatman) column chromatography after cleavage with *Eco*RI and used in all subsequent analyses. Molecular cloning of the unintegrated form of SSAV genome in Charon 16A has been described (2). A 2.6-kb fragment derived from SSAV DNA after digestion with *Kpn* I encompassing the *env* gene was used for the sequence analysis.

DNA Sequence Analysis. The nucleotide sequence was determined by the procedure of Maxam and Gilbert (8). DNA fragments were obtained by using various restriction endonucleases and were labeled either at their 5′ end by using [γ-^{32}P]ATP (Amersham; 3,000 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq) and polynucleotide kinase (P-L Biochemicals) (8) or at their 3′ end by using cordycepin 5′-[α-^{32}P]triphosphate (Amersham; 3,000 Ci/mmol) and terminal deoxynucleotidyltransferase (P-L Biochemicals) according to Roychoudhury and Wu (9). End-labeled DNA fragments were digested with appropriate restriction endonucleases (New England BioLabs), isolated by agarose or polyacrylamide gel electrophoresis, and used for sequence analysis.

Preparation of Antisera. Pentadecapeptides based on predicted amino acid sequences were obtained from Peninsula Laboratories (San Carlos, CA). About 100 μg of the appropriate peptide was coupled with thyroglobulin (10) and administered to rabbits intraperitoneally at 14-day intervals. Animals were bled 1 wk after each injection. The effectiveness of the immune response was monitored by the ability of serum from sequential blood samples to precipitate the ^{125}I-labeled peptide.

Immunoprecipitation Analysis. Subconfluent cultures (about 10^7 cells per 10-cm Petri dish) were labeled for 3 hr at 37°C with 4 ml of methionine-free Dulbecco's modified Eagle's minimal essential medium containing 100 μCi of [^{35}S]methionine (1,200 Ci/mmol; Amersham) per ml. Radiolabeled cells were lysed, immunoprecipitated, and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis as described (11).

RESULTS

Primary Sequence of the SSV Genome. The primary nucleotide sequence of the integrated SSV genome was determined according to the partial chemical degradation method of Maxam and Gilbert (8). The sequences of both strands were determined for most of the genome, and known restriction cleavage sites were confirmed by sequence analysis. The complete sequence of SSV along with its flanking rat cellular sequences are presented in Fig. 1. The 5,779-nucleotide SSV genome contained 504-base-pair long terminal repeats (LTRs). The woolly monkey cell-derived *onc* sequence, v-*sis*, which is essential for SSV transformation (6), encompassed 1,006 base pairs and was localized between positions 3,811 and 4,817 in the SSV genome (Fig. 1). Some of the salient features of the viral genome are summarized in Fig. 2.

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Abbreviations: SSV, simian sarcoma virus; SSAV, simian sarcoma-associated virus; kbp, kilobase pair(s); Mo-MuLV, Moloney murine leukemia virus; LTR, long terminal repeat.

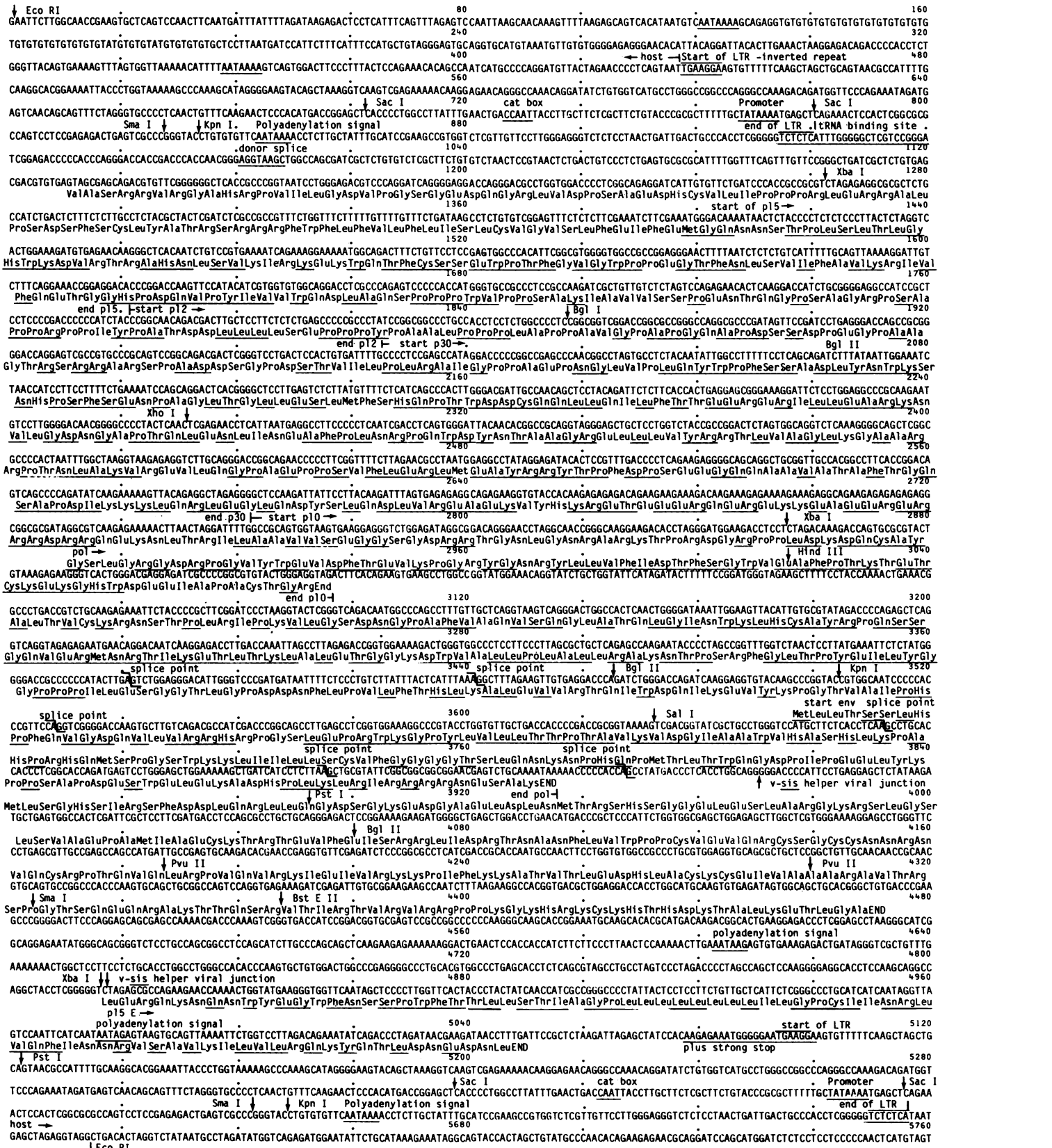

FIG. 1. Complete nucleotide sequence of the proviral SSV genome. The sequence proceeding in the 5′ to 3′ direction has the same polarity as SSV genomic RNA. Every 20th nucleotide is marked by a dot. The sequence is corrected from that reported previously (12) by addition of cytosine residues at positions 3,702, 4,068, and 4,101 and a guanosine residue at position 4,202. The amino acid sequence deduced from the open reading frame is given either above or below the nucleotide sequence. The major structural features of the genome are indicated. Amino acid residues in common with those of murine leukemia virus (Mo-MuLV) are underlined.

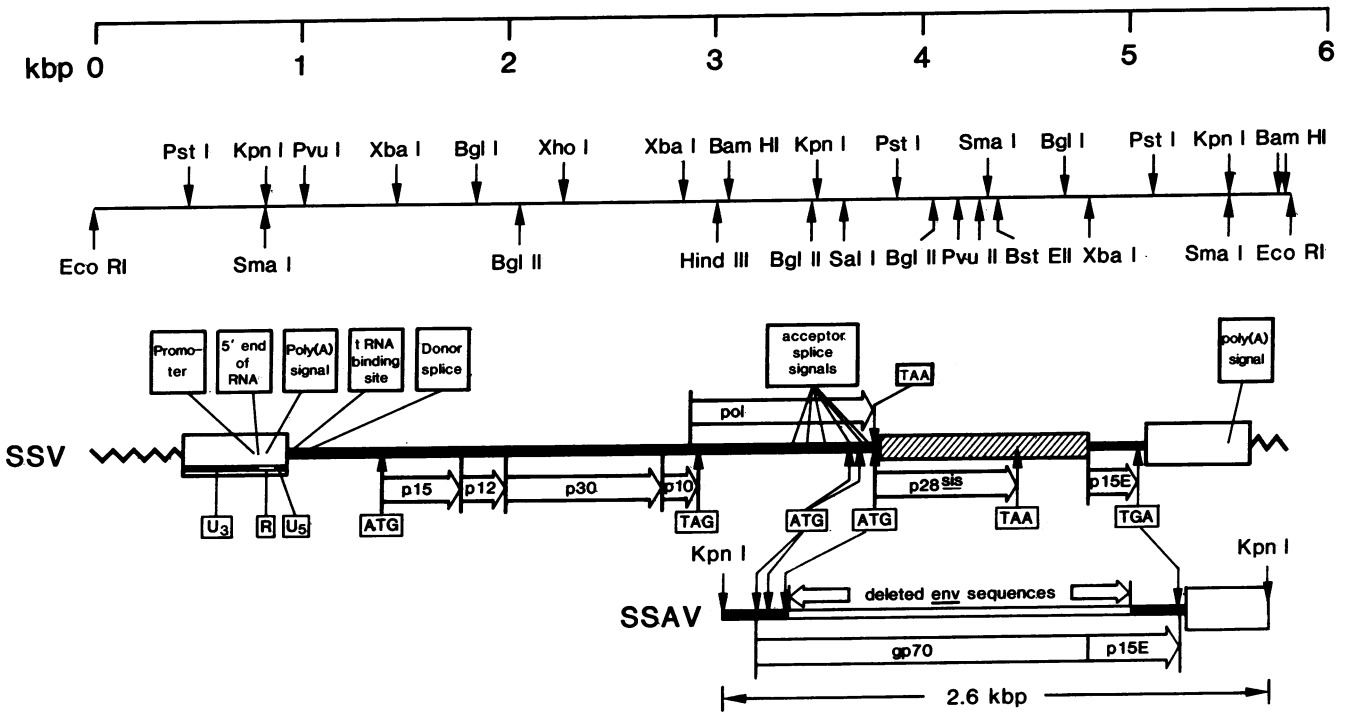

FIG. 2. Summary of the major structural features of the SSV genome. Important features of the SSV genome, including the opening reading frames, possible signals for promoter, polyadenylylation, and donor and acceptor splice signals are indicated. The precise location of substitution of SSAV *env* gene sequences with v-*sis* in the SSV genome is also shown.

Sites of SSV Integration. Rat cellular sequences immediately flanking the SSV LTRs were found to contain a four-nucleotide direct repeat sequence, T-A-A-T, confirming previous findings of duplication of a short stretch of sequences at the site of retrovirus integration (13, 14). In the 5'-flanking rat cellular sequence, two promoter-like sequences and a 42-nucleotide-long sequence consisting of 21 repeats of the dinucleotide T-G were detected. The role of these sequences with respect to SSV integration is not known.

The SSV LTRs, like previously studied avian and mouse type C viral LTRs (7, 13–20), contain signals for the initiation and termination of viral RNA transcription and resemble prokaryotic transposable elements (Figs. 1 and 2). Certain retroviral sequences are known to be critical in processes related to reverse transcription. These include a prolyl tRNA binding site (21) that followed the 5' LTR at positions 942–960 and the purine-rich plus-strand stop DNA (19) that preceded the 3' LTR (Figs. 1 and 2).

Identification of SSAV Coding Sequences Within the SSV Genome. It is known that the respective gene products of SSAV share crossreacting antigenic determinants with those of MuLV (22, 23). Moloney murine sarcoma virus and Mo-MuLV are the only retroviruses for which complete nucleotide sequence data are available (24–26). In an attempt to precisely localize SSAV coding regions within the SSV genome, the primary nucleotide and amino acid sequences of the viral RNA strand of SSV were compared with those of Mo-MuLV in all three reading frames by using the ALIGN program (27).

As shown by the underlined homologous amino acid sequences in Fig. 1, there was an excellent correlation between the nucleotide and amino acid sequences in the SSAV-derived *gag*, *pol*, and *env* coding regions of SSV and those of Moloney-MuLV. Moreover, the predicted sequence of SSV protein p30 (Fig. 1) matched well with the partial amino acid sequence data available for SSAV p30 (28). Sequence comparison further showed that (*i*) the entire SSAV *gag* gene was present in the SSV genome and was localized at positions 1,398–2,933. (*ii*) The *pol* gene had undergone a single large deletion at its amino terminus, leaving a stretch of 882 nucleotides that corresponded to the carboxyl terminus of this gene. Sequence analysis of Mo-MuLV has shown that the *pol* gene open reading frame is overlapped at its 3' end with the amino terminal region of the *env* gene coding sequence (26). By sequence comparison with Mo-MuLV, the SSAV-derived *pol* sequence of SSV also included the overlapping amino terminus of the SSAV *env* gene, whose reading frame extended into v-*sis*. (*iii*) To the right of the 3' v-*sis* SSAV junction, it was possible to identify sequences that showed significant homology with the carboxyl-terminal region of the MuLV *env* gene (Fig. 2). In fact, sequence analysis of the SSAV genome showed that the nucleotides at positions 4,818–5,047 of the SSV genome (Fig. 1) were derived from the carboxyl-terminal region of the SSAV genome coding for protein p15E (Fig. 2). These findings together with the previously reported observation that the U5 and R region of LTRs and tRNA binding sites show close sequence homology among mammalian retroviruses (7) indicate that functionally important sequences of these viruses have been well conserved.

Role of SSAV Sequences in the Transcription of v-*sis*. We had earlier identified four possible splice-acceptor signals in helper viral sequences upstream from v-*sis* (12). In the present study, we identified two additional splice-acceptor signals. One of these corresponded to the acceptor splice signal proposed to be involved in the generation of Mo-MuLV subgenomic *env* mRNA (26). The location, at positions 1,001–1,008, of the putative splice donor signal for the mRNA is shown in Fig. 2. SSV-transformed nonproducer cells contain a single subgenomic RNA of about 2.7 kb. This RNA has been shown to hybridize with LTR and v-*sis* probes but not with SSAV *gag*-specific probes (unpublished data). Such a RNA could be generated by using the acceptor and donor signals identified here.

A promoter-like sequence was identified at position 3,771. We have found that subgenomic SSV DNA clones that lack the

5′ LTR retain transforming activity but with somewhat reduced efficiency (unpublished data). Thus, transcription can be initiated in the absence of the 5′ LTR and donor splice signals, and an internal promoter-like signal could also initiate transcription of the SSV transforming gene.

Role of SSAV Sequences in the Translation of v-*sis*. Examination of the region of the SSV genome to which its transforming gene has been localized (6) indicated a long open reading frame. This reading frame initiated at position 3,657 within SSAV sequences to the left of the 5′-SSAV v-*sis* junction and terminated with an ochre codon within v-*sis* at position 4,470 (Fig. 2). By sequence comparison with Mo-MuLV, the open reading frame to the left of v-*sis* was identified as initiating from the amino-terminal region of the SSAV *env* gene. Sequence comparison with Mo-MuLV revealed 37% homology with SSV in this region. Sequence analysis of molecularly cloned SSAV confirmed that this region codes for the amino-terminal sequences of its *env* gene. The open reading frame of the SSAV *env* gene was identical from position 3,657 to position 3,810 at the 5′ end of the *sis* open reading frame (Fig. 1). This stretch of sequences contained two more ATG codons in the same reading frame. One of these corresponded to the ATG codon that has been proposed as the initiator codon of the Mo-MuLV *env* gene product (26). This initiator codon could be used for synthesis of the v-*sis* gene product from a spliced mRNA analogous to that used for the *env* gene product (29, 30).

If the v-*sis* gene product is synthesized from the ATG at position 3,657, a protein of M_r approximately 33,000 containing 271 amino acids, will result. A v-*sis* gene product synthesized from the second or third ATG would result in a protein of M_r 30,000 or 28,000, respectively. We have recently identified a M_r 28,000 protein in SSV-transformed cells by using antibody directed against the predicted amino acid sequence of v-*sis* at its amino-terminal region and found that antibodies directed against the SSAV *env* gene product failed to precipitate this protein (6). These findings are consistent with translation of the v-*sis* gene product initiating at the third ATG. However, rapid processing of a larger protein cannot be excluded.

Demonstration that the SSV Transforming Gene Product (p28^{sis}) Is Encoded Within the v-*sis* Open Reading Frame. By construction of SSV deletion mutants, we have shown that the SSV transforming region encompassed v-*sis* together with 345 nucleotides to the left and 305 nucleotides to the right of v-*sis* (6). To conclusively establish that p28^{sis} is encoded within the v-*sis* open reading frame and to confirm our predicted nucleotide sequence of the SSV transforming gene, we took two approaches. Within the predicted amino acid sequence, methionine residues were localized at nucleotide positions 3,840, 3,936, and 4,023. The largest cyanogen bromide cleavage product would have a M_r of 18,000. SSV-transformed cells were radiolabeled for 3 hr with cysteine. Cell extracts were immunoprecipitated with anti-amino-terminal peptide serum and subjected to cyanogen bromide cleavage and polyacrylamide gel electrophoresis analysis. We observed a M_r 18,000 protein (data not shown), a result consistent with the predicted structure of this protein.

We also prepared antibody to a pentadecapeptide derived from the carboxyl terminus of the predicted protein. This peptide was localized within sequences of v-*sis* at position 4,425–4,469 in the SSV genome. As shown in Fig. 3, antibody to both the amino- and the carboxyl-terminal peptides of the v-*sis* open reading frame detected p28^{sis} in SSV-transformed but not in uninfected cells. In competition experiments, precipitation of this protein was specifically inhibited by the appropriate unlabeled peptide. Moreover, neither antisera specifically precipitated any protein in cells infected with SSAV alone (data not shown). All of these findings confirm the validity of our predicted amino acid sequence for p28^{sis} and verify that this protein is encoded within the v-*sis* open reading frame.

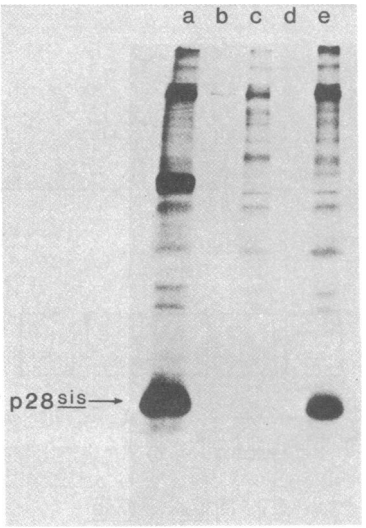

FIG. 3. *In vivo* detection of the v-*sis* translational product by immunoprecipitation analysis. Subconfluent cultures (approximately 10^7 cells per 10-cm Petri dish) were labeled for 3 hr at 37°C with 4 ml of methionine-free Dulbecco's modified Eagle's minimal essential medium containing 100 μCi of [^{35}S]methionine (1,200 Ci/nmol; Amersham) per ml. Radiolabeled cells were lysed with 1 ml of 10 mM sodium phosphate, pH 7.5/100 mM NaCl/1% Triton X-100/0.5% sodium deoxycholate/0.1 mM phenylmethylsulfonyl fluoride per Petri dish, the lysates were clarified at 100,000 × *g* for 30 min, and 200-μl aliquots were incubated with 4 μl of antiserum for 60 min at 40°C. Immunoprecipitates were recovered with the aid of *Staphylococcus aureus* protein A bound to Sepharose beads (Pharmacia) and analyzed by electrophoresis in NaDodSO$_4$/14% polyacrylamide gels as described (11). Lanes: a, SSV clone 11-transformed nonproducer NRK cells with antiserum directed against the amino-terminal v-*sis* peptide (position 3,813–3,857); b, uninfected NRK cells with antiserum against the amino-terminal peptide; c, SSV clone 11-transformed nonproducer NRK cells with preimmune rabbit serum; d, uninfected NRK cells with antiserum directed against the carboxyl-terminal v-*sis* peptide (position 4,425–4,469); e, SSV clone 11-transformed nonproducer NRK cells with antiserum against the carboxyl-terminal v-*sis* peptide.

DISCUSSION

We have studied the structural organization of the SSV genome at the primary nucleotide level. These studies have enabled us to identify signals for the transcription and translation of SSV-encoded proteins, including the putative product of the SSV transforming gene. By Southern blotting and heteroduplex analysis, we have shown that SSV arose in nature by deletion of approximately 3.7 kbp of SSAV sequences and substitution of about 1 kbp of cellular sequences localized toward the 3′ end of the genome (2). In the present study, sequence analysis of the entire SSV genome and comparison with the sequence of the SSAV *env* gene as well as the known sequence of Mo-MuLV enabled us to precisely identify the structure of the SSV genome as 5′-*gag*-Δ*pol*-Δ*env*-*sis*-Δ*env*-3′.

A number of transforming retroviruses synthesize their transforming proteins by means of *gag-onc* polyproteins, the initiator codon of which is contributed by the amino-terminal region of the helper viral *gag* gene. The long open reading frame within the SSV transforming region was found to initiate within SSAV sequences that code for its *env* gene. In fact, the initiator codon for the SSAV *env* gene could also serve for synthesis of the SSV transforming protein by using the same reading frame. Analo-

gous findings have been reported for Moloney murine sarcoma virus, in which the first five amino acids of the putative transforming protein are contributed by helper viral sequences derived from the amino terminus of the Mo-MuLV *env* gene (24, 25). Thus, transforming proteins of many retroviruses appear to be hybrid proteins in which *gag* or *env* genes initiate their translation.

We have no direct evidence that the SSV transforming protein functions as an *env-sis* hybrid protein. In fact, antibodies to SSAV virion proteins do not precipitate the SSV transforming gene product, $p28^{sis}$ (6), and the protein itself has an apparent molecular weight that can almost entirely be accounted for by the v-*sis* open reading frame. Thus, whether the *env* precursor region of a hybrid protein is rapidly processed or the gene product is synthesized from an initiating codon closer to v-*sis* is not yet known.

Our present findings indicate four nucleotide changes from our previously reported sequence of v-*sis* (Fig. 1) (12). These changes do not alter the size of the predicted protein nor the major portion of the molecule. However, the predicted amino acid sequence of the central region of the molecule is changed. The protein remains hydrophilic in nature with no transmembrane-specific amino acid sequences at either terminus. However, the hydrophobic region in the center of the molecule is shorter than predicted earlier (12). Antibodies directed against peptides derived from both the amino- and the carboxyl-terminal regions of the v-*sis* open reading frame precipitated $p28^{sis}$, helping to confirm our sequence of this open reading frame as well as localizing the coding region for $p28^{sis}$ to its *sis* sequence. As our previous studies have shown that this region is essential for SSV transformation (6), $p28^{sis}$ is the product of the SSV transforming gene. Partial characterization of $p28^{sis}$ has shown that the molecule is not phosphorylated and lacks detectable protein kinase activity (6). In these aspects, $p28^{sis}$ is distinguishable from a number of other retrovirus transforming gene products (31–41). Thus, the molecular mechanism by which SSV transforms cells is likely to differ from those of other retroviral *onc* gene products with known functional activities.

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1. Thielen, G. J., Gould, D., Fowler, M. & Dungworth, D. L. (1971) *J. Natl. Cancer Inst.* **47**, 881–889.
2. Robbins, K. C., Devare, S. G. & Aaronson, S. A. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2918–2922.
3. Gelmann, E. P., Wong-Staal, F., Kramer, R. A. & Gallo, R. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3373–3377.
4. Wong-Staal, F., Favera, R. D., Gelmann, E. P., Manzari, V., Szala, S., Josephs, S. F. & Gallo, R. C. (1981) *Nature (London)* **294**, 273–275.
5. Robbins, K. C., Hill, R. L. & Aaronson, S. A. (1982) *J. Virol.* **41**, 721–725.
6. Robbins, K. C., Devare, S. G., Reddy, E. P. & Aaronson, S. A. (1982) *Science* **218**, 1131–1133.
7. Devare, S. G., Reddy, E. P., Law, J. D. & Aaronson, S. A. (1982) *J. Virol.* **42**, 1108–1113.
8. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
9. Roychoudhury, R. & Wu, R. (1980) *Methods Enzymol.* **65**, 43–62.
10. Kagan, A. & Glick, M. (1979) in *Methods of Hormone Radioimmunoassay*, eds. Jaffe, B. M. & Behrman, H. R. (Academic, New York), pp. 328–329.
11. Barbacid, M., Lauver, A. V. & Devare, S. G. (1980) *J. Virol.* **33**, 196–207.
12. Devare, S. G., Reddy, E. P., Robbins, K. C., Andersen, P. R., Tronick, S. R. & Aaronson, S. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3179–3182.
13. Shimotohno, K., Mizutani, S. & Temin, H. (1980) *Nature (London)* **285**, 550–554.
14. Dhar, R., McClements, W. L., Enquist, L. W. & Vande Woude, G. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3937–3941.
15. Ju, G. & Skalka, A. M. (1980) *Cell* **22**, 379–386.
16. Reddy, E. P., Smith, M. J., Canaani, E., Robbins, K. C., Tronick, S. R., Zain, S. & Aaronson, S. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5234–5238.
17. Sutcliffe, J. G., Shinnick, T. M., Verma, I. M. & Lerner, R. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3302–3306.
18. Van Beveren, C., Goddard, J. C., Berns, A. & Verma, I. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3307–3311.
19. Swanstrom, R., DeLorbe, W. J., Bishop, J. M. & Varmus, H. E. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 124–128.
20. Rushlow, K. E., Lautenberger, J. A., Reddy, E. P., Souza, L. M., Baluda, M. A. Chirikjian, J. G. & Papas, T. S. (1982) *J. Virol.* **42**, 840–846.
21. Taylor, J. M. (1977) *Biochim. Biophys. Acta* **473**, 57–71.
22. Hino, S., Stephenson, J. R. & Aaronson, S. A. (1975) *J. Immunol.* **115**, 922–927.
23. Barbacid, M., Stephenson, J. R. & Aaronson, S. A. (1977) *Cell* **10**, 641–648.
24. Reddy, E. P., Smith, M. J. & Aaronson, S. A. (1981) *Science* **214**, 445–450.
25. Van Beveren, C., Van Straaten, F., Galleshaw, J. A. & Verma, I. M. (1981) *Cell* **27**, 97–108.
26. Shinnick, T. M., Lerner, R. A. & Sutcliffe, J. G. (1981) *Nature (London)* **293**, 543–548.
27. Dayhoff, M. D. (1976) *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Washington, D.C.), Vol. 5, Suppl. 2.
28. Oroszlan, S., Copeland, T., Smythers, G., Summers, M. R. & Gilden, R. V. (1977) *Virology* **77**, 413–417.
29. Mellon, P. & Duesberg, P. H. (1977) *Nature (London)* **270**, 631–634.
30. Rothenberg, E., Donoghue, D. J. & Baltimore, D. (1978) *Cell* **13**, 435–451.
31. Collett, M. S. & Erikson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2021–2024.
32. Levinson, A. D., Oppermann, H., Levintow, L., Varmus, H. E. & Bishop, J. M. (1978) *Cell* **15**, 561–572.
33. Barbacid, M., Beemon, K. & Devare, S. G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5158–5162.
34. Feldman, R. A., Hanafusa, T. & Hanafusa, H. (1980) *Cell* **22**, 757–765.
35. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
36. Kawai, S., Yoshida, M., Segawa, K., Sugiyama, R., Ishizaki, R. & Toyoshima, K. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6199–6203.
37. Pawson, T., Guyden, J., Kung, T., Radke, K., Gilmore, T. & Martin, G. S. (1980) *Cell* **22**, 767–775.
38. Van de Ven, W. J. M., Reynolds, F. H. & Stephenson, J. R. (1980) *Virology* **101**, 185–197.
39. Witte, O. M., Dasgupta, A. & Baltimore, D. (1980) *Nature (London)* **283**, 826–831.
40. Neil, J. C., Ghysdael, J. & Vogt, P. K. (1981) *Virology* **109**, 223–228.
41. Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. (1979) *Virology* **96**, 64–79.