Configuration and Terminal Sequences of the Simian Varicella Virus Genome

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The simian varicella virus (SVV) genome is a linear DNA molecule consisting of a unique short (Uₚ) and a unique long (Uₗ) region. The Uₚ is bounded by internal (IR) and terminal inverted repeats and invets such that virion DNA contains equimolar amounts of two genome isomers. We have sequenced the right and leftward termini and the Uₗ-IR junction region of the SVV genome. A sequence motif common to other herpesviruses, consisting of A and T residues surrounded by G+C-rich regions, was found near the rightward terminus of the SVV genome. Sequence analysis showed no repeats surrounding the Uₗ region of the genome. Nucleic acid hybridization and polymerase chain reaction (PCR) amplification using primers from the right and leftward ends of the SVV genome indicated that the Uₗ region inverts. PCR amplification also showed that, compared with virion DNA, SVV genomes with connected termini are increased in infected cell DNA, suggesting the presence of circular or concatameric genomic molecules. © 1995 Academic Press, Inc.

INTRODUCTION

Simian varicella virus (SVV) causes an exanthematous disease of nonhuman primates. Numerous reports comparing genome structure and composition (Clarke et al., 1992; Gray et al., 1992; Pumphrey and Gray, 1992; Gray and Oakes, 1984), immunological crossreactivity (Fletcher and Gray, 1992; Felsenfeld and Schmidt, 1977, 1979), pathogenesis (Duealand et al., 1992; Wenner et al., 1977), and virus latency (Mahalingam et al., 1991, 1992) indicate that SVV infection of primates parallels varicella zoster virus (VZV) infection in humans.

SVV virion DNA is linear, consisting of a unique long (Uₗ) region of 110 kb (Clarke et al., 1992; Gray et al., 1992) and a unique short (Uₚ) region of 4.9 kb (Fletcher and Gray, 1993). The Uₚ is bounded by internal and terminal repeat (IR and TR) sequences and inversion of the Uₚ results in two equimolar isomers of SVV DNA, similar to other herpesviruses (Clarke et al., 1992; Gray et al., 1992). The Uₗ regions of VZV (Davison, 1984; Davison and Wikle, 1983; Kinchington et al., 1985; Ruyechar et al., 1985; Straus et al., 1981) and herpes simplex virus (HSV) (Davison and Wikle, 1981) also invert. Herein, we report the nucleotide sequence of the genome termini and the Uₗ-IR junction of SVV virion DNA and examine inversion of the Uₗ region. We have also used PCR amplification to compare the configuration of the SVV genome in the virion and in SVV-infected cells.

MATERIALS AND METHODS

Cells and virus

The Delta herpesvirus strain of SVV (Ayers, 1971) was propagated in the BSC-1 line of African green monkey kidney cells (Clarke et al., 1992). DNA was extracted from infected cells (Sambrook et al., 1989) and SVV virions (Clarke et al., 1992).

Construction of plasmid pSLT

The PCR amplification product generated from primers 1 and END (Fig. 1) was cloned into the TAIi cloning vector (Invitrogen Corp., San Diego, CA). It was then shuttled into pPstO, which contains the SVV DNA fragment PstI O (Clarke et al., 1992). The resulting plasmid, pSLT, contained one copy of template DNA for primers Pst A and B and one copy of template DNA for primers 1 and END.

DNA electrophoresis, electrotransfer, and hybridization conditions

After restriction enzyme digestion, SVV DNA was electrophoresed through 0.8% agarose gels and immobilized onto Zeta probe membranes using the Trans blot electrophoretic transfer system (Bio-Rad, Richmond, CA). PCR amplification products were electrophoresed through 1 or 2% agarose gels and similarly transferred. Nick-translated hybridization probes were prepared from electroluted EcoRI/BamHI subfragments of SVV EcoRI E DNA (Rigby et al., 1977) using [³²P]dCTP (Amersham, Arlington Heights, IL). SVV-specific oligonucleotides (Operon Technologies, Inc., Alameda, CA) internal to the amplifi-
culation primers were end-labeled with $^{32}$P]ATP (ICN Radiochemicals, Irvine, CA).

PCR amplification

Stock solutions of oligonucleotide primers (Operon Technologies, Inc.) were diluted in sterile water to $5 \mu M$ for use in PCR reactions. PCR was performed as described (Mahalingam et al., 1991) with a denaturing step at 94°C for 2 min, an annealing step at 55°C for 2 min, and an elongation step at 72°C for 3 min, for a total of 34 cycles. As a control, no DNA was included in each experiment.

Sequencing

Sequencing was performed using a Sequenase Version 2.0 kit (United States Biochemicals, Cleveland, OH) with SP6, T7 (Promega, Madison, WI), and SVV-specific oligonucleotide (Operon Technologies, Inc.) primers. The sequenced products were run on 1% Long Ranger acrylamide gels (T. Baker, Inc., Phillipsburg, NJ) containing 14 M urea.

RESULTS

Sequence of the SVV termini and IR--U$_{IR}$ junction of the SVV genome

Sequence of the rightward terminus of the SVV genome. The rightward EcoRI fragment of the SVV genome is EcoRI L (Clarke et al., 1992; Gray et al., 1992). We cloned EcoRI L by electroleuting it from an agarose gel, blunt-ending it with T4 DNA polymerase, and ligating it into Smal-digested pGEM 3Z. The resulting plasmid was sequenced using T7 and SP6 primers to generate sequence from both sides of EcoRI L DNA. These sequences were compared with the sequence of EcoRI E DNA which spans the IR--U$_{IR}$ junction (Fig. 1). The sequence of EcoRI L DNA adjacent to the SP6 promoter was identical to the sequence of EcoRI E DNA within the IR region (results not shown). The sequence of EcoRI L DNA adjacent to the T7 promoter therefore represented the rightward terminal sequences of the SVV genome. Sequencing of multiple clones revealed a motif of A and T residues surrounded by G+C-rich regions (Fig. 2A) in the rightward end of the SVV genome.

Sequence of the IR--U$_{IR}$ junction of the SVV genome. Based on sequence of the rightward terminus of SVV DNA, a primer (primer 2) was chosen which allowed sequencing across the U$_{IR}$--IR junction (Fig. 1). The sequence obtained (Fig. 2B) matched that of the rightward end of the SVV genome and continued into sequences at the leftward end of the U$_{IR}$; a run of 9 T residues lies 23 bp into the U$_{IR}$.

Sequence of the leftward terminus of the SVV genome. EcoRI I is the leftward EcoRI fragment of the SVV genome (Clarke et al., 1992; Gray et al., 1992). Repeated attempts to clone EcoRI I were unsuccessful. However, the presence of a BclI site ~200 bp from the left terminus of the SVV genome was identified by cloning and sequencing the PCR product generated after amplification of SVV-infected cell DNA with primers 1 and END2 (Fig. 1). Electroleuted EcoRI I was digested with BclI, blunted-ended with T4 DNA polymerase, and ligated into the Smal site of pGEM 3Z. The resulting insert was sequenced, using SP6 and T7 primers. The leftward terminus of the SVV

![Diagram](image)

FIG. 1. Diagram of the SVV genome. The U$_{IR}$, U$_{IR}$, IR, and TR regions and the EcoRI E, L, and I fragments (Clarke et al., 1992) are marked. Also shown are relevant EcoRI (E), BclI (B), and BglII (Bg) restriction endonuclease sites and the position and direction of the PCR/sequencing primers (END, END2, 1, 2, PacI, and PacII).

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A

5'----GCGGT AGCCAGCTG TCCAGAAGAC ATCCAGGATC CGTCAAGCTC 3'----CCCG CAGCGCTG ACGCTGACG TACTGAGT

B

5'----TGCGT AGGCGACAC TGCGAAGAC ATCCAGGATG CGTTCAAGCTC 3'----AACG TCCGCTGAT CAGCTGACG TACTGAGT

C

5'----ATAAT ATATGTCG CGATAATGAC GCGCAAGCTC TACGTGACG TACTGAGT

FIG. 2. Sequences of the rightward end (A), the IR--U$_{IR}$ junction region (B), and the leftward end (C) of the SVV genome. The ends of the genome (*) and the IR--U$_{IR}$ junction are marked. Potentially important motifs consisting of A and T residues flanked by G+C-rich regions, and strings of T residues are underlined.
genome was identified by comparison to the sequence of the product generated after PCR amplification of SVV-infected BSC-1 DNA using primers 1 and END (Figs. 1 and 3). The sequence of the leftward end of the SVV genome is shown in Fig. 2C. The leftward end of the SVV genome, and hence the leftward end of the U_L, is not homologous to the rightward end of the U_L, indicating that the SVV genome does not have repeat sequences surrounding the U_L. There is a run of 5 T residues 31 bp internal to the leftward end of the genome.

Sequencing across the TR-U_L junction of the SVV genome

The PCR product resulting from amplification of SVV-infected BSC-1 cell DNA using primers 1 and END (Fig. 1) was cloned and sequenced (Fig. 3). The sequence obtained starts in the leftward end of the U_L, extends through the leftward terminus, and continues into the rightward terminus of the TR. A G+C bp not detected in the sequences of the nonadjacent termini is located between the sequences for the left and rightward ends of the SVV genome.

Inversion of the SVV U_L DNA region

Electroeluted, nick-translated probes EBa1 and EBa2 (Fig. 4) were hybridized to an SVV EcoRI DNA digest (Fig. 5). Probe EBa2 identified bands corresponding to SVV EcoRI L and E DNA. A fainter band ~7 kbp in size was also seen. Hybridization with probe EBa1 identified EcoRI E DNA and again a fainter band ~7 kbp. The weak 7-kbp fragments most likely represent the 7.6-kbp (in the case of EBa2) and the 7.1-kbp (in the case of EBa1) DNA fragments resulting from inversion of the U_L (Fig. 4). The presence of circular genomes in the SVV virion DNA preparation would result in a 7.6-kbp band after hybridization with EBa2, however, circularization would not account for a 7.1-kbp band after hybridization with EBa1. The relative intensities of the 7.1- and 7.6-kbp bands indicate that if circular genomes are present they are much less abundant than genomes containing an inverted U_L. The other faint bands correspond to SVV EcoRI fragments I and J (Clarke et al., 1992) and most probably represent nonspecific hybridization.

PCR amplification of SVV virion and SVV-infected BSC-1 DNA

PCR amplification was conducted on SVV virion DNA, SVV-infected BSC-1 DNA, and psLT DNA (a plasmid containing one copy of template DNA for each of the two primer sets used). The first primer set was chosen from the leftward and rightward end sequences of the SVV genome (primers END and 1, Fig. 1), and the second set was chosen from within the U_L (primers PstA and B, Fig. 1). Sequence information for primers PstA and B was obtained by sequencing the plasmid pPstO (Clarke et al., 1992).

To ensure that primers 1 and END were derived from
infected cells showed that the fraction of SVV genomes with a configuration allowing primers 1 and END to amplify, compared to those allowing primers PstA and B to amplify, is larger in SVV-infected cell DNA than in SVV virion DNA (Fig. 7). The formation of SVV genome forms circles or the presence of genome concatemers in infected cells most likely explains these results.

**DISCUSSION**

Analysis of the right and leftward ends and the U<sub>1</sub>–IR junction of the SVV genome both confirmed the absence of repeat sequences surrounding the U<sub>1</sub> and identified sequence motifs found at the termini of other herpesvirus genomes. Hybridization studies showed that virion DNA contains SVV genomes with an inverted U<sub>1</sub>. Terminal primers were selected which would amplify SVV DNA only if the virus genome were in a circular or concatemeric form or if the U<sub>1</sub> region of the genome had inverted. These terminal primers, in conjunction with primers from within the U<sub>1</sub>, were used for PCR analysis of DNA from SVV virions and from cells productively infected with SVV. Compared to SVV virion DNA, infected cell DNA contains a higher proportion of SVV genomes with connected termini, consistent with the presence of SVV DNA in a circular or concatemeric configuration.

The detection of an additional G+C base pair across the ligated ends of SVV DNA, compared to the sequences obtained by direct cloning of the genome termini, indicates that the SVV genome termini contain an unpaired 3' nucleotide, since the action of T4 DNA polymerase would have removed an unpaired 3', but not an unpaired 5', nucleotide during cloning. The presence of an unpaired, complementary 3' nucleotide is thought to facilitate HSV genome circularization (Mocarski and Rolz).
man, 1982a; Davison, 1984; Tamashiro and Spector, 1986; Hammerschmidt et al., 1988, Chowdhury et al., 1989), which occurs in infected cells (Ben-Porat and Veach, 1980; Davison and Wilkie, 1983; Jean et al., 1977, Garber et al., 1993), prior to DNA replication. The increased percentage of SVV DNA molecules with adjacent ends in infected cells, compared to virion DNA, is consistent with postinfection circularization.

After circularization, limited DNA replication and inversion of repeat sequences may occur. HSV DNA inversion may be mediated by virus-specific factors that act upon terminally redundant "a" sequences common to the termini and the U1-U3 joint region (Mocarski and Roizman, 1982b), but no evidence exists for site-specific segment inversion of VZV DNA. Nevertheless, a sequence motif composed of A and T residues and flanked by G+C-rich sequences has been found near the end of the VZV TR and IR regions (Davison, 1984), and could act as a specific recombination site. The rightward terminus of SVV DNA contains this consensus sequence. It is also present in HSV-1 and 2 (Davison and Wilkie, 1981), in EHV-1 (Chowdhury et al., 1999), in BHV (Hammerschmidt et al., 1988), and PRV (Harper et al., 1986), all of which invert their U3 regions. The sequence may also function as a cleavage recognition site.

A greater proportion of SVV genomes with connected termini is present in infected cells compared to virion DNA. This suggests that, like HSV DNA (Ben-Porat and Rixon, 1979; Jacob et al., 1979), SVV DNA replicates by a rolling circle mechanism, producing genome concatamers. These must be precisely cleaved, indicating endonuclease action at a specific nucleotide sequence, to produce unit-length genomes that are packaged into virions. In addition to the consensus sequence at the rightward end of the SVV genome, the leftward end contains a string of 5 Ts, 31 bp from the terminus which may correspond to strings of As or Ts found in other herpesviruses: PRV (9As, 3Ts) (Harper et al., 1988); BHV (10As, 4Ts) (Hammerschmidt et al., 1988); EHV-1 (7As, 5Ts) (Chowdhury et al., 1989); VZV (10As) (Davison, 1984); and HSV-1 and HSV-2 (5As) (Davison and Wilkie, 1981), which are all located 31–42 bps in from the leftward end of the viral genomes.

PCR amplification using primers from the right and left termini of the SVV genome suggests that the U1 region of SVV DNA invets in 1–20% of cases. A similar fraction (about 5%) of VZV DNA molecules also contain an inverted U1 (Davison, 1984). It has been proposed that cleavage of VZV DNA normally occurs at the novel U1–TR joint, formed by head-to-tail arrangements of genomes produced by rolling circle replication. Occasionally, cleavage also occurs at the normal U1–IR joint. This would explain both major and minor VZV genome arrangements. If cleavage is site-specific, then part of the recognition signal must lie within the U1.

In conclusion, we have identified terminal sequence motifs in SVV DNA that are conserved in other herpesviruses and have detected SVV genomes with an inverted U1 in virion DNA despite the absence of inverted repeats surrounding the U1 region. We have also demonstrated, by PCR amplification, an increased percentage of SVV genomes with adjacent ends in infected cell DNA, an observation consistent with postinfection genome circularization. We are currently extending these findings to determine the configuration of SVV DNA in latently infected monkey ganglia.

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