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Structure and Heterogeneity of the *a* Sequences of Human Herpesvirus 6 Strain Variants U1102 and Z29 and Identification of Human Telomeric Repeat Sequences at the Genomic Termini

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The unit-length genome of human herpesvirus 6 (HHV-6) consists of a single unique component (U) bounded by direct repeats DR_L and DR_R and forms head-to-tail concatemers during productive infection. *cis*-elements which mediate cleavage and packaging of progeny virions (*a* sequences) are found at the termini of all herpesvirus genomes. In HHV-6, DR_L and DR_R are identical and *a* sequences may therefore also occur at the U-DR junctions to give the arrangement aDR_La-U-aDR_Ra. We have sequenced the genomic termini, the U-DR_R junction, and the DR_R.DR_L junction of HHV-6 strain variants U1102 and Z29. A $(GGGTTA)_n$ motif identical to the human telomeric repeat sequence (TRS) was found adjacent to, but did not form, the termini of both strain variants. The DR_L terminus and U-DR_R junction contained sequences closely related to that of the well-conserved herpesvirus packaging signal C_n-G_n-N_n-G_n (*pac-1*), followed by tandem arrays of TRSs separated by single copies of a hexanucleotide repeat. HHV-6 strain U1102 contained repeat sequences not found in HHV-6 Z29. In contrast, the DR_R terminus of both variants contained a simple tandem array of TRSs and a close homolog of a herpesvirus *pac-2* signal (GC_n-T_n-GC_n). The DR_R.DR_L junction was formed by simple head-to-tail linkage of the termini, yielding an intact cleavage signal, *pac-2*.x.*pac-1*, where x is the putative cleavage site. The left end of DR was the site of intrastrain size heterogeneity which mapped to the putative *a* sequences. These findings suggest that TRSs form part of the *a* sequence of HHV-6 and that the arrangement of *a* sequences in the genome can be represented as aDR_La-U-*a*-DR_Ra.

Human herpesvirus 6 (HHV-6) is a recently isolated member of the herpesvirus family (45). Primary infection with HHV-6 is the cause of exanthem subitum in infants (60), and seroconversion in most individuals occurs within the first year of life (5, 40). In common with other herpesviruses, HHV-6 can be reactivated following immunosuppression and frequently has been recovered from immunocompromised individuals, including those with AIDS (8, 17, 31, 39, 54, 59). HHV-6 isolates have recently been segregated into two groups on the basis of differences in molecular and biological properties (1). Variant A viruses are characterized by HHV-6 U1102 (17) and GS (45), and variant B viruses are characterized by strain Z29 (31). A restriction map of HHV-6 U1102 has been constructed (33). The genome consists of a single unique sequence (U) of 141.5 kb flanked by identical direct repeats (DR_L and DR_R) of 10 kb (33). The structure of the Z29 isolate of HHV-6 is similar to that of strain U1102 (29), and both strain variants show an unstable size heterogeneity mapping to the DR (29, 33). Nucleotide sequencing studies of HHV-6 (9, 18, 19, 21, 24, 28, 32, 38, 55, 56) have established that the virus is most closely related to human cytomegalovirus (HCMV).

a sequences are found at the genomic termini of all herpesviruses and are required in *cis* for cleavage and packaging of progeny genomes into capsids (2, 11, 35–37, 47, 51, 52, 58). *a* sequences are also found at the junctions of the long (L) and short (S) components of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) (13, 36, 37) and HCMV (35, 53) and are believed to mediate inversion of the L and S components (44). The HSV-1 *a* sequence contains the unique sequences U_b and U_c linked by a variable number of DR elements (13, 36, 37), yielding the canonical structure DR1-U_b-$DR2_n$.$DR4_m$-U_c-DR1. U_b and U_c have been shown to contain well-conserved signals, designated *pac-1* and *pac-2*, which are necessary for cleavage and packaging of HSV-1 virion DNA (14). The unit-length genomes of HHV-6 form head-to-tail concatemers during lytic infections (29, 33). The terminal DRs in HHV-6 are identical, and *a* sequences may therefore also occur at the U-DR junctions, giving the arrangement aDR_La-U-aDR_Ra. In this paper, we present the nucleotide sequence of the U-DR_R and DR_R.DR_L junctions and genomic termini of HHV-6. Elements characteristic of *a* sequences occur at both the genomic termini and U-DR_R junction of HHV-6. The putative *a* sequences of both strains of HHV-6 contain well-conserved *pac-1* and *pac-2* homologies, but neither terminus contains an intact cleavage signal. A $(GGGTTA)_n$ motif, which is identical to human telomeric repeat sequences (TRSs) (34), lies adjacent to both ends of the unit-length genome of HHV-6.

MATERIALS AND METHODS

Cells and viruses. HHV-6 U1102 was grown in peripheral blood lymphocytes obtained from healthy donors. Peripheral blood lymphocytes were isolated by centrifugation on Ficoll-Paque gradients (Pharmacia) and grown in RPMI 1640 medium supplemented with 10% fetal calf serum, phytohemagglutinin (5 μg/ml), and antibiotics (penicillin, 10 μg/ml; streptomycin, 20 μg/ml). HHV-6 Z29 was grown in phytohemagglutinin-stimulated primary cord blood mononuclear cells as described elsewhere (16). Viruses were propagated by

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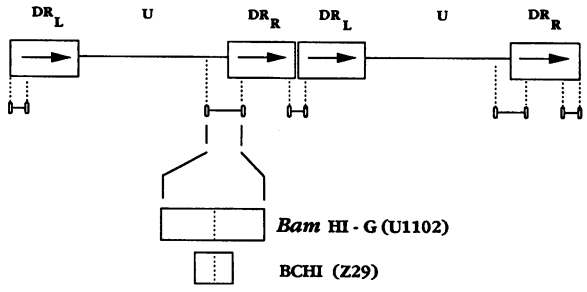

FIG. 1. Regions of HHV-6 selected for nucleotide sequence analysis. The unit-length genome of HHV-6 consists of a single unique sequence (U) of 145 kb flanked by identical DRs (DR_L and DR_R) 10 kb in length (29, 33). Two genomes are shown here linked head-to-tail in a concatemer. The regions sequenced were the left terminus (i.e., the left end of DR_L), the right terminus (i.e., the right end of DR_R), the U-DR_R junction, and the DR_R.DR_L junction linking unit-length genomes. The positions of *Bam*HI-G in strain U1102 and clone BCHI in strain Z29 are illustrated, and the U-DR_R junctions are indicated by vertical dashed lines.

cocultivation of infected cells (10^6/ml) at a 1:8 ratio with uninfected cells and were harvested when a peak cytopathic effect was observed.

Virus DNA preparation and Southern blotting. HHV-6-infected cells were harvested, washed in phosphate-buffered saline, and treated with sodium dodecyl sulfate (SDS)-proteinase K followed by phenol-chloroform extraction. DNA was ethanol precipitated, suspended in double-distilled water, and digested with selected restriction enzymes. Restriction fragments were resolved on 0.6% agarose gels in 89 mM Tris-borate–2 mM EDTA and blotted onto nylon membranes in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Filters were then prehybridized in 6× SSC–1% SDS–0.5% dried milk and hybridized with a $(GGGTTA)_3$ probe end labelled with [^{32}P]dATP.

Isolation of terminal fragments. Terminal fragments were isolated in two ways. Restriction fragments containing the genomic termini of HHV-6 U1102 (*Sal*I-O and *Sal*I-P) (33) were excised from 0.6% agarose gels, extracted with Geneclean according to the manufacturer's instructions, blunt-ended with T4 DNA polymerase, and cloned into *Sma*I-cut pBluescript. Terminal fragments from HHV-6 Z29 virion DNA (16) were isolated essentially as described by Davison (12). Briefly, intact virion DNA was sonicated and blunt ended with T4 DNA polymerase prior to cloning into sequencing vectors. Clones for subsequent sequencing were then selected by colony hybridization with a $(GGGTTA)_3$ probe end labelled with [^{32}P]dATP.

DNA sequencing and analysis. An approximately 9-kb *Bam*HI clone (*Bam*HI-G) (33), which spans the U-DR_R junction of HHV-6 U1102 (33) (Fig. 1), was selected for analysis. Random fragments of *Bam*HI-G DNA were produced according to the methods described by Bankier et al. (3), and single-stranded template was sequenced with the dideoxynucleotide chain termination method (46) as modified by Bankier et al. (3). Regions of compression in the *Bam*HI-G sequence were resolved with water-jacketed sequencing gels run at 65°C. A 1.9-kb *Bam*HI fragment (BCHI), which spans the U-DR_R region of HHV-6 Z29 (29), was subcloned from clone λ 155 (a generous gift of P. E. Pellett) and sequenced with Sequenase version 2.0 (United States Biochemical) or TaqTrack (Promega) to extend oligonucleotide primers. The terminal fragments of HHV-6 U1102 and Z29 were sequenced by double-stranded methods with Sequenase version 2.0 or a *Taq* Dyedeoxy Terminator Cycle Sequencing kit (Applied Biosystems Ltd.), used according to the manufacturers' instructions on an ABI automated DNA sequencer. PCR products (see below) were sequenced on an ABI automated sequencer. Sequence data were assembled with the DBAUTO (48) and DBUTIL (49) programs and analyzed with the program ANALYSEQ (50) or GCG (15).

Amplification of DR.DR junctions. Sequence derived from the termini of the unit-length genomes of HHV-6 U1102 and HHV-6 Z29 was used to select primers for amplification of a DR_R.DR_L junction (Fig. 1) by PCR. The primers oriented rightward from the right end of DR_R were 5′ CGGACCGAA GACAGAGGGC 3′ and 5′ TAACCCATCCCCCAACGCGC 3′ for HHV-6 U1102 and HHV-6 Z29, respectively. The primers oriented leftward from DR_L were 5′ CGTAATTTT TAAAACGCGAGG 3′ (HHV-6 U1102, yielding an 800-bp product) and 5′ TTAGGGTTAATACCCCCCTT 3′ (HHV-6 Z29, yielding a 155-bp product). The positions of these primers in the sequence of the respective termini are indicated below. PCR was carried out with 50 ng of infected-cell DNA as template and Promega *Taq* polymerase and buffers (Mg, 1.5 mM final concentration). PCR products were precipitated in 26.2% polyethylene glycol solution (26.2% polyethylene glycol 8000, 6.6 mM $MgCl_2$, 0.6 M NaOAc), resuspended in double-distilled water, and sequenced (~0.5 μg of DNA template per reaction) with the *Taq* Dyedeoxy Terminator Cycle Sequencing kit according to the manufacturer's instructions.

Nucleotide sequence accession number. The sequences reported here appear in the EMBL, GenBank, and DDJB sequence data bases under accession numbers L22335, L22336, and L22337.

RESULTS

The nucleotide sequence of BCHI, together with that of the corresponding region of *Bam*HI-G, is shown in Fig. 2. Both fragments are predicted by restriction mapping to span the U-DR_R junction of the HHV-6 isolate from which they were derived (29, 33) and thus should contain sequences also found at the left genomic terminus, i.e., the left end of DR (Fig. 1). The length of *Bam*HI-G determined by sequencing was 8 kb, approximately 1 kb shorter than estimates based on the restriction map of HHV-6 U1102 (33). The nucleotide sequence of the left end of *Bam*HI-G, which lies in the unique component of HHV-6 U1102, had a mean G+C content of 35%, whereas the sequence of the right end, presumed to lie within DR, had a mean G+C content of 65% (data not shown).

Repetitive sequences. The sequences of both BCHI and *Bam*HI-G contained sets of reiterated sequences which are shown in detail in Fig. 3. BCHI contained eight tandem arrays of a $(GGGTTA)_n$ motif identical to the human TRS (34) (shown on the positive strand as TAACCC in Fig. 2 and 3), separated by a single copy of a hexanucleotide sequence, TAGGTC. We have designated these repeats DR2 and DR3 because they followed a unique region corresponding to the U_c component of the HSV-1 *a* sequence (described below). The $(TAACCC)_n$ array was followed by tandem repeats of the closely related sequence TAGCCC, which we have also designated DR2. The structure of the corresponding region in *Bam*HI-G was more complex. The sequences TAACCC and TAGCCC occurred in the same tandem array separated, as in BCHI, by the hexanucleotide TAGGTC. In addition, *Bam*HI-G contained a number of other randomly distributed repeats differing from the TAACCC motif at a single base, two copies of TAACCAAAACATYAACCC (DR4), and complete and partial copies of CCC/GTAGCCAAAGTACTAACCC TAR (DR5). A 63-bp imperfect DR in BCHI was poorly conserved in the sequence of *Bam*HI-G.

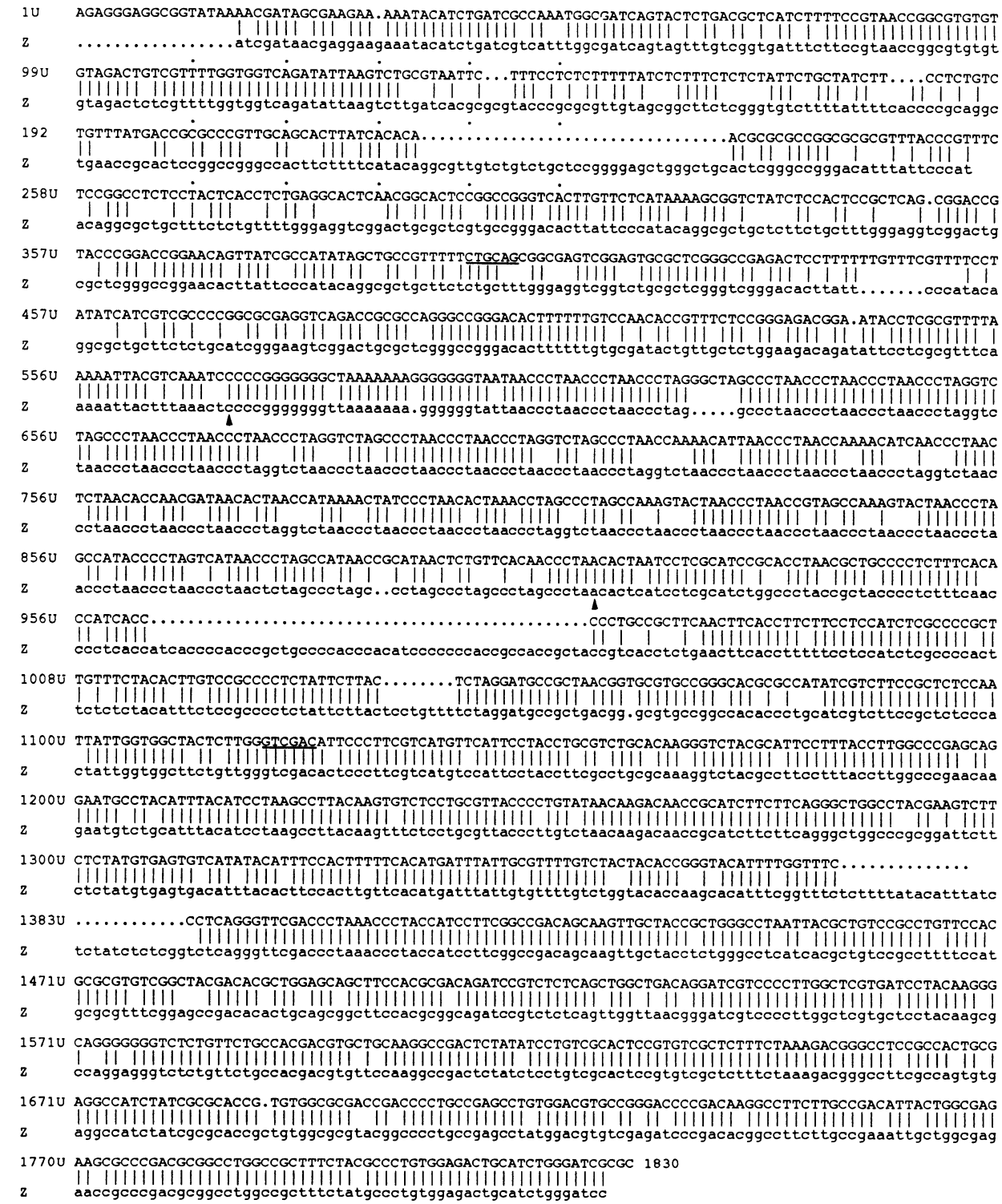

FIG. 2. Nucleotide sequence of the BCHI subclone (Z) of clone λ 155 of HHV-6 strain Z29 (29) shown together with the corresponding region of the *Bam*HI-G clone (U) of HHV-6 U1102 (33). The sequence is shown in the 5′-to-3′ direction and is numbered according to the sequence of *Bam*HI-G. Sequence alignment was carried out with the GCG program (15). The sequence shown spans the U-DR_R junction of both strain variants of HHV-6. The *Pst*I site (CTGCAG, position 399 to 404U) and the *Sal*I site (GTCGAC, position 1120U to 1125U) which flank the region of heterogeneity in HHV-6 U1102 (see text) are indicated. The boundaries of the putative *a* sequence are indicated by the arrowheads.

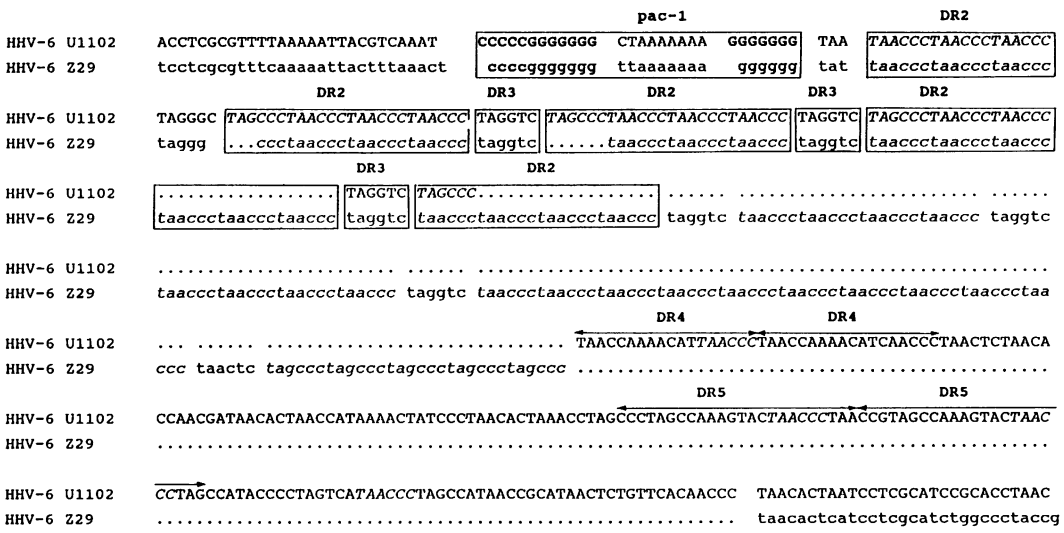

FIG. 3. Reiterated sequences at the U-DR_R junctions of HHV-6 U1102 and Z29. The first position shown is the base identified as the terminal nucleotide at the left end of the DR (see text). A motif conforming closely to the well-conserved herpesvirus *pac-1* signal (C_n-G_n-N_n-G_n) is indicated. This signal in HSV-1 occurs within U_b of the canonical *a* sequence DR1-U_b-$DR2_n$.$DR4_m$-U_c-DR1 (13, 36, 37), and conventional nomenclature has been used to identify subsequent repeats in the putative *a* sequence of HHV-6. The TRS motif GGGTTA, shown here on the positive strand as TAACCC, has accordingly been designated DR2, and the intervening repeat TAGGTC has been designated DR3. Repeat sequences common to both viral strains have been boxed, and gaps in one sequence relative to the other are indicated by dots. TRS (DR2) sequences are shown in italics, and occur elsewhere in the sequence of HHV-6 U1102. Two additional repeats, designated DR4 and DR5, are found in HHV-6 U1102. Alignments were carried out by visual inspection.

Identification of the genomic termini. In order to identify and characterize the termini of the HHV-6 genome, terminal fragments obtained from two sources were sequenced: (i) the *Sal*I-O and *Sal*I-P fragments containing termini of HHV-6 U1102 (33) and (ii) clones selected from a library of sonicated fragments of HHV-6 Z29 by colony hybridization with a $(GGGTTA)_3$ probe. All clones were blunt ended with T4 DNA polymerase prior to cloning into sequencing vectors, thus preserving the 5′-terminal base but removing any 3′ extension. The sequence of each terminus is shown in Fig. 4. DNA used to isolate the terminal clones was prepared from stocks of virus different from those of the DNA used to isolate junctional fragments. $(TAG/ACCC)_n$.TAGGTC.$(TAG/ACCC)_n$ motifs from the left end of DR_R were present at copy numbers (8 to >30) in terminal clones different from those in clones derived from the U-DR_R junction of the same strain of HHV-6 (data not shown), suggesting heterogeneity of this region in vivo. The primary structure of the right terminus differed from that of the left terminus. The right termini of HHV-6 U1102 and HHV-6 Z29 each contained a simple tandem array of $(GGGTTA)_n$ (Fig. 4). The number of reiterations of GGGTTA ranged from 15 to >60 and varied both in different right-terminal clones from the same virus isolate and, sometimes, on repeat sequencing of the same clone, indicating that the apparent difference in copy number may be, in part, a sequencing artifact. No other repeat sequences were identified. Comparison of the left terminus and U-DR_R with that of the right terminus showed that, with the exception of the $(GGGTTA)_n$ motif, which was arranged differently at each end of the genome, no sequences occurred at both termini. No equivalent of the HSV-1 DR1 element (13, 36, 37) was identified. The left terminus and U-DR_R junction, however, contained sequences conforming closely to the conserved *pac-1* (C_n-G_n-N_n-G_n) signal found in the U_b element of the HSV-1 *a* sequence and identified in other herpesviruses (2, 11, 13, 35–37, 52, 53) (Fig. 3 and 4). The right terminus did not contain an identifiable *pac-1* homology but did contain sequences closely resembling the *pac-2* (GC_n-T_n-GC_n) signal found in the U_c region of a number of other *a* sequences (2, 11, 13, 36, 37) (Fig. 4). The proximity of the *pac-1* and *pac-2* homologies to the termini in HHV-6 is consistent with that found in other herpesviruses (Fig. 4).

Sequence of the DR.DR junction. Amplification of the DR_R.DR_L junctions of both HHV-6 Z29 and U1102 resulted in a single amplification product. The sequences of the DR.DR junctions, together with the positions of the PCR primers used to generate the amplification products, are illustrated in Fig. 4. DR.DR junctions are formed by head-to-tail linkage of DR_R and DR_L to yield an arrangement, *pac-2*.x.*pac-1*, where x is the putative cleavage site (Fig. 4). The sequences of junction 1 in HHV-6 Z29 and U1102 were each obtained from a number of separate amplification products and are consistent with the presence of a single 3′ base extension at each terminus of HHV-6 Z29 (which had been removed from terminal clones during the cloning procedure). In contrast, the sequence of junction 2 of HHV-6 Z29, which was also obtained from a number of PCR products, has a single base insertion at the DR.DR junction. Sequences from the right termini of both isolates varied at a number of positions, whereas sequences from the left termini did not (data not shown). We have, at present, no explanation for these anomalies, but note that sampling of junctions by PCR may amplify sequences which are not present in most virions.

Heterogeneity of junctional and terminal fragments. To determine whether the variable number of $(TAG/ACCC)_n$.TAGGTC.$(TAG/ACCC)_n$ repeats found in terminal and junctional sequences were an artifact of bacterial cloning and sequencing or a consequence of intrastrain size heterogeneity in viral stocks, Southern blots of DNA from cells infected with HHV-6 U1102 were hybridized with a $(GGGTTA)_3$ oligonucleotide (Fig. 5A). DNA was digested with five restriction

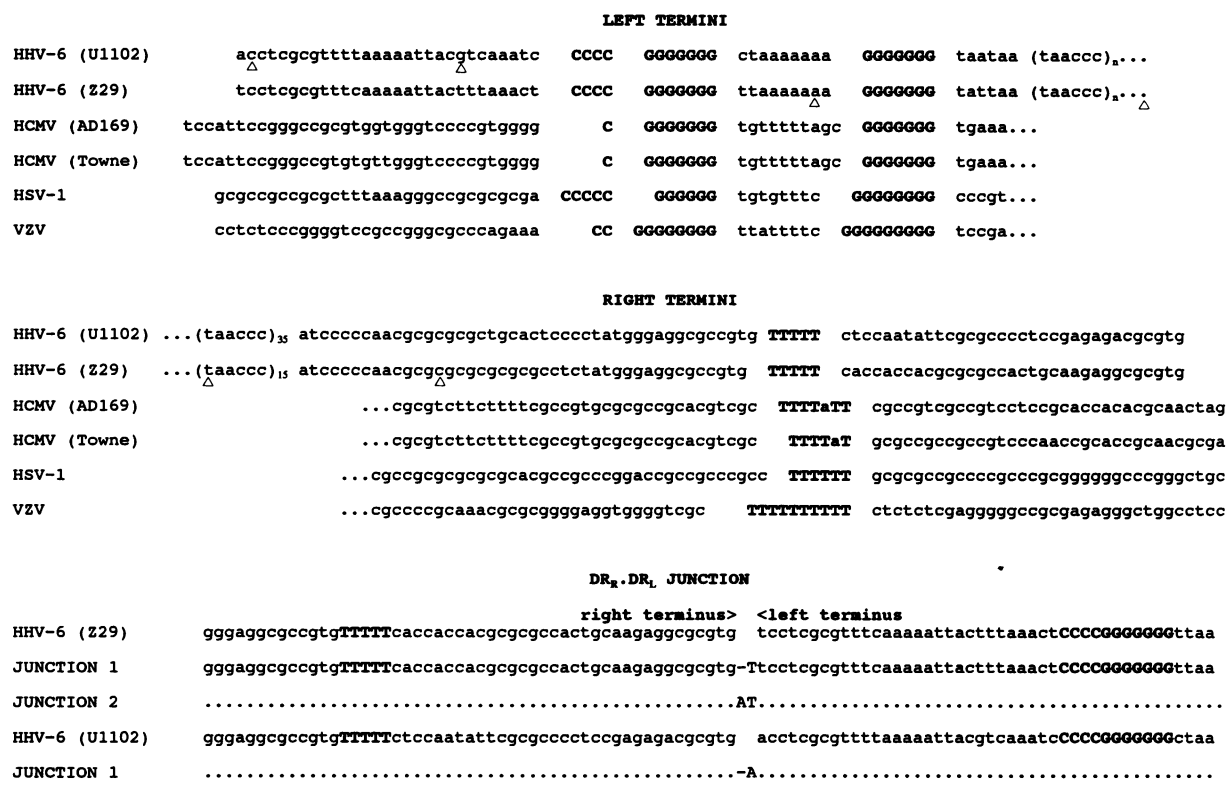

FIG. 4. Structure of the genomic termini of HHV-6 U1102 and Z29, illustrated together with the corresponding termini of HCMV AD169 (53) and Towne (35), HSV-1 (36, 37), and varicella-zoster virus (VZV) (11). *pac-1* sequences (C_n-G_n-N_n-G_n) are indicated in left-terminal sequences, and sequences closely related to the herpesvirus *pac-2* signal (GC_n-T_n-GC_n) are indicated in right-terminal sequences. Terminal nucleotides were identified after blunt ending with T4 DNA polymerase, and any 3′ base extension will have been removed. The structure of the DR_R.DR_L junction is also shown. Bases which are present in junctional fragments, but not in the sequences of termini isolated with T4 DNA polymerase, are shown in uppercase letters. The boundaries of primers used to amplify the DR.DR junction are indicated by the open triangles. Primers oriented leftward from the left terminus [5′ CGTAATTTTTAAAACGCGAGG 3′ for HHV-6 U1102 and 5′ TTAGGGTTAATACCCCCCTT 3′ for HHV-6 Z29, where the 5′ three bases are the first three nucleotides of the second $(TAACCC)_n$ repeat] are shown on the opposite strand. The sequence used to derive the primer oriented rightward from the right terminus of HHV-6 U1102 (5′ CGGACCGAAGACAGAGGGC 3′) lies upstream of the sequence illustrated.

enzymes (*Sal*I, *Sma*I, *Hin*dIII, *Pst*I, and *Bam*HI). The $(GGGTTA)_3$ probe recognized restriction fragments which formed both the right terminus, i.e., *Sal*I-O, and the left terminus, i.e., *Sal*I-P, of the unit-length genome (Fig. 5A and B, respectively). In addition, the $(GGGTTA)_3$ probe hybridized to fragments which crossed the U-DR_R junction (*Sal*I-G) and the DR_L-U junction (*Sal*I-L) (Fig. 5A and B, respectively). These experiments therefore confirmed that the GGGTTA motif occurred at both termini and both U-DR junctions of HHV-6 U1102. The $(GGGTTA)_3$ probe detected intrastrain size heterogeneity of up to 1.5 kb in the left end of DR which was present in fragments from both the left terminus and the U-DR_R junction (fragments designated h in Fig. 5A and het in Fig. 5B). In contrast, no heterogeneity was detected at the right terminus or DR_L-U junction, although a small difference in the number of copies of $(GGGTTA)_n$ would not have been detected. Furthermore, PCR with primers spanning TRSs from the right terminus consistently produced a single band of the expected size, whereas PCR with primers spanning the left-terminal TRS produced a variable ladder of products (55a).

The *Bam*HI-G fragment sequenced in this study is 8 kb in length and is therefore a het fragment (Fig. 5). Comparison of restriction sites in the sequence of *Bam*HI-G with the map of HHV-6 U1102 (33) indicates that this size difference lies between the *Pst*I and *Sal*I sites indicated in Fig. 2 (data not shown) and therefore maps to the region of the *a* sequence. The size heterogeneity noted at the left end of DR was unstable with serial passage of HHV-6 U1102 and is consistent with observations made during restriction mapping of this strain variant of HHV-6 (33). Analysis of the sequence of *Bam*HI-G for the presence of open reading frames by using the program ANALYSEQ (50) indicated that the sequence illustrated in Fig. 2 is open to translation on both strands. The potential polypeptides encoded are not homologous to identified herpesvirus proteins (data not shown), but we do not exclude the possibility that the U-DR_R junction in HHV-6 codes for protein. Heterogeneity in the copy number of hexanucleotide repeats would therefore lead to the production of a polymorphic protein.

DISCUSSION

The genome of HHV-6 consists of a single unique component flanked by identical DRs to yield the arrangement DR_L-U-DR_R (29, 33). This structure is unique among human herpesviruses and resembles that of channel catfish virus (10) and equine herpesvirus 2 (7). Unit-length genomes of HHV-6 form head-to-tail concatemers during lytic infection (29, 33). Elements required for the cleavage and packaging of progeny genomes (*a* sequences) must lie at DR_R.DR_L junctions in

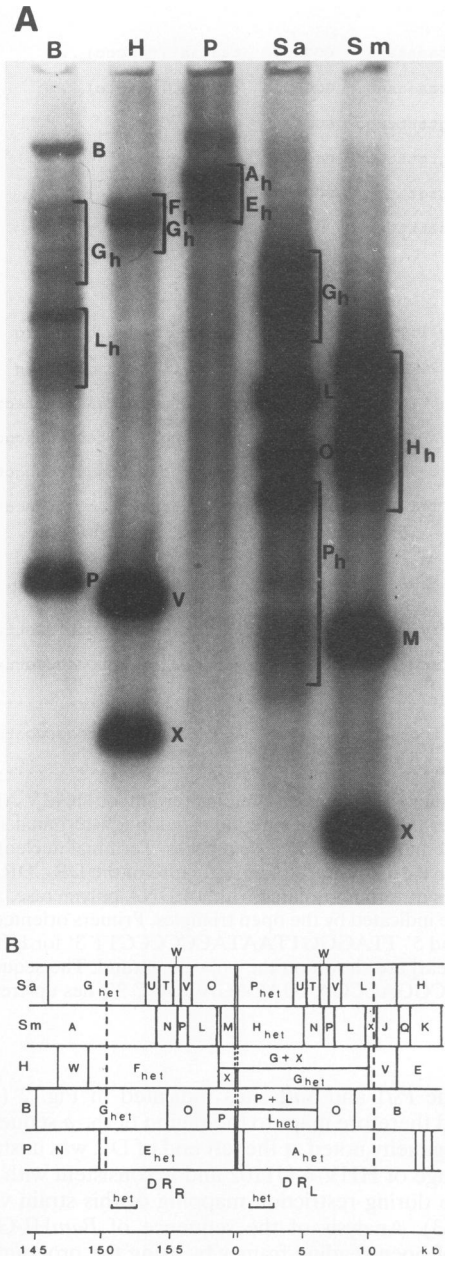

FIG. 5. (A) Results of hybridization of a $(GGGTTA)_3$ probe end labelled with [^{32}P]dATP to a blot of HHV-6 U1102-infected-cell DNA digested with enzymes *Sal*I (Sa), *Sma*I (Sm), *Hin*dIII (H), *Bam*HI (B), and *Pst*I (P). (B) Restriction map of the DR_R.DR_L junction in a concatemeric junction between two unit-length genomes of HHV-6 U1102 DNA, shown for the same enzymes. The restriction map is based on that of Martin et al. (33), modified according to the nucleotide sequence of *Bam*HI-G. The sizes of individual fragments are not shown but correspond closely to those given by Martin et al. (33). The junction between the right end of the left genome (DR_R) and the left end of the right genome (DR_L) is shown by the double solid lines. The U-DR junctions are indicated by the dashed lines. Fragments described with a single letter lie either within the DR or are linked to the ends of the unique component of the genome. Fragments created by head-to-tail linkage across the DR_R.DR_L junction are also indicated for *Hin*dIII and *Bam*HI, but for reasons of clarity, the DNA selected for use in the experiment illustrated contained predominantly virions and not concatemers. Fragments which show size heterogeneity are designated het in the map of the DR_R.DR_L junction (B) and h in fragments detected by hybridization with the $(GGGTTA)_3$ probe (A).

concatemers and at the U-DR junctions and termini of virion DNA. The organization of reiterated sequences at the U-DR_R junction and termini of HHV-6 U1102 and HHV-6 Z29 does not conform to the canonical DR1-U_b-$DR2_n$.$DR4_m$-U_c-DR1 arrangement found in the *a* sequences of HSV-1 (13, 36, 37). In particular, no DR1 element could be identified in either strain variant of HHV-6 and the boundaries of the *a* sequence could not be accurately determined. Both strain variants of HHV-6, however, contained motifs closely resembling the conserved herpesvirus *pac-1* and *pac-2* signals (2, 11, 13, 36, 37, 53). The putative *a* sequences of both strain variants of HHV-6 have two striking features. First, the *pac-1* and *pac-2* signals are present at opposite ends of the genome and neither terminus has an intact cleavage signal. Second, a $(GGGTTA)_n$ motif identical to the human TRS (34) lies adjacent to both termini.

Role of *pac-1* and *pac-2* signals. Herpesviruses can be divided into two groups on the basis of the arrangement of *a* sequences at their genomic termini. Members of the first group, characterized by HSV-1 and -2 (13, 36, 37, 51) and HCMV (35, 53), generally possess an *a* sequence at both ends of the unit-length genome. In HSV-1, the substrate for cleavage and packaging is a junction in which the *pac-1* (in U_b) and *pac-2* (in U_c) signals flank the cleavage site in DR1 in the orientation *pac-2*.DR1.*pac-1* (14, 57). This occurs within a double *a* junction, i.e., DR1-U_b-$DR2_n$.$DR4_m$-U_c-DR1-U_b-$DR2_n$.DR_m-U_c-DR1 (13, 36, 37). Junctions containing a single *a* sequence can also be processed to make ends by a mechanism which involves initial amplification of the *a* sequence to bring the *pac* signals into the correct orientation on either side of the cleavage site (14, 57). The second group of herpesviruses is represented by varicella-zoster virus (11) and bovine herpesvirus 1 (23). In these viruses, *pac-1* and *pac-2* homologies are present at opposite ends of the genome and fusion of the termini during replication generates junctions in which *pac-1* and *pac-2* signals flank the cleavage site in the correct orientation. Amplification of *a* sequences in these viruses has not been described. The arrangement of *pac-1* and *pac-2* signals at the termini of the unit-length genome of HHV-6 conforms to the second group of viruses. Fusion of DR_R and DR_L will yield an arrangement, *pac-2*.x.*pac-1*, where x is the cleavage site. The sequence of the DR.DR junction in HHV-6 Z29 confirms that this arrangement occurs at concatemeric junctions in infected-cell DNA. The requirement for fusion of the termini to generate an intact cleavage signal means that only DR.DR junctions, and not U-DR junctions, can serve as a substrate for cleavage, thus ensuring production of virions with the correct genomic architecture. Both the primary structure of the *pac* signals in HHV-6 and their distances from the termini are very similar to those found in other herpesviruses (Fig. 4). This observation provides support for a measurement mechanism for the generation of genomic termini and also suggests that cleavage and packaging of HHV-6 virion DNA occur by mechanisms conserved throughout the herpesvirus family.

Role of the $(GGGTTA)_n$ motif. The $(GGGTTA)_n$ motif is identical to the TRSs which form the ends of human chromosomes (34). These repeats are the substrate for a novel class of DNA polymerase, known as telomere terminal transferase (telomerase), which acts principally to ensure complete replication of the linear chromosome (4). The role of TRSs in replication is critically dependent on the formation of a terminal overhang by the G-rich strand of the $(GGGTTA)_n$

The $(GGGTTA)_3$ probe recognized restriction fragments which formed both genomic termini, i.e., *Sal*I-O and *Sal*I-P, and fragments which spanned both U-DR junctions, i.e., *Sal*I-G and *Sal*I-L (see text).

sequence (4). In addition, TRSs mediate the segregation of linear chromosomes during cell division (4) and have been shown to improve the efficiency of segregation of circular plasmids during cell division in *Saccharomyces cerevisiae* (30). It is interesting that the general structure of human telomere-associated DNA has many similarities to that of herpesvirus *a* sequences (6). A $(GGGTTA)_n$ motif was found to be responsible for cross-hybridization between the genomes of HHV-6 and the avian herpesviruses Marek's disease virus and herpesvirus of turkeys (26). $(GGGTTA)_n$ sequences have subsequently been localized to an *a*-like sequence at the L-S junction of Marek's disease virus (25) and cross-hybridize with restriction fragments present at the L-S junction and termini of herpesvirus of turkeys (43). These studies did not, however, determine the structure of the viral termini. We have shown that the $(GGGTTA)_n$ repeats in HHV-6 lie close to, but do not form, the ends of the viral chromosome. This finding, together with the identification of conventional herpesvirus *pac* signals, mitigates strongly against a role for telomerase in the replication of HHV-6 during lytic infection. HHV-6 persists in peripheral blood lymphocytes of seropositive adults (22) and has been shown to establish latent infection in cells of the monocyte-macrophage lineage (27). It is attractive to postulate that TRSs in HHV-6 confer the capacity to persist as a linear minichromosome. In HHV-6, however, TRSs lie 60 to 80 bp from the termini, and it is difficult to envisage how these sequences could protect or replicate the ends of a linear viral chromosome. Further, herpesviruses which establish latency in dividing cells normally do so as plasmid episomes, which replicate via a plasmid origin (20, 42, 61). TRSs in *S. cerevisiae* dramatically increase the efficiency of plasmid segregation during cell division (30), and we hypothesize that TRSs in HHV-6, Marek's disease virus, and herpesvirus of turkeys play a similar role in maintenance of the viral chromosome during latent infection in dividing cells. It is of great interest that we have also found the $(GGGTTA)_n$ motif at the U-DR_R junction of HHV-7 (15a). It will be interesting to determine whether this group of herpesviruses also possess a plasmid origin of replication. The roles of TRS and TRS binding proteins (4) in these viruses are the subject of current studies.

Heterogeneity of the termini of HHV-6. The size heterogeneity previously noted in HHV-6 strain variants U1102 and Z29 is unstable in culture and has been mapped to the left end of the DR (29, 33). A $(GGGTTA)_3$ probe detects this heterogeneity in both HHV-6 U1102 (Fig. 5) and HHV-6 Z29 (41). This finding, together with the detection of a variable copy number of complex repeats in the sequence of the left terminus and U-DR_R junction and mapping of het sequences to the U-DR junction in *Bam*HI-G, suggests that the heterogeneity is at least in part a property of the left-terminal *a* sequence and may therefore be generated by the cleavage and packaging reaction. Further work is in progress to map the limits of this heterogeneity and to determine whether multiple copies of the intact *a* sequence can be detected in some samples of HHV-6. The presence of distinct *pac-1* and *pac-2* signals in herpesvirus *a* sequences implies a polarity in the process of cleavage and packaging (14, 57) which is consistent with heterogeneity of a single terminus. The molecular events which generate such heterogeneity may be intrinsic to the cleavage and packaging reaction in HHV-6 and merit further investigation.

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